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COMMONWEALTH BUREAU OF ANIMAL HEALTH

VETERINARY REVIEWS AND ANNOTATIONS

Vol. 1] October, 1955 [Part II

A Discussion on Atrophic Rhinitis of Swine with Special Reference to Recent Work in Canada

BY

RONALD GWATKIN, D.V.M., D.V.Sc.
Animal Pathology Division, Canada Department of Agriculture, Animal Diseases Research Institute, Hull, Quebec.

INTRODUCTION

The purpose of this review is to consider some of the more recent work on infectious atrophic rhinitis. The writer has drawn heavily on published and unpublished work done at the Animal Diseases Research Institute, Hull, Quebec (1) in order to present a picture of this disease as it appears to him at the present time. No attempt has been made to include all the papers that have been published on this subject in the bibliography because such a procedure would increase volume without adding much to the scientific knowledge of this disease.

While the intention of the writer is to reconstruct the situation as it appears at present, some reference to earlier reports should be made as an introduction to the subject. For this purpose an English translation of a paper by Thunberg & Carlström (2) in 1940 has been freely drawn upon. In this paper dealing with sneezing sickness or atrophic rhinitis of swine in Sweden the authors considered that the first report on this condition was by Franque in 1842. From the clinical description he gave they considered that the condition was the same as that with which they were dealing. They could find no further references in the literature until a paper by Schneider in 1878. That author regarded the disease as an inborn malformation of a hereditary nature and considered the nasal catarrh to be a secondary phenomenon. Bang in 1915 took more or less the same point of view as Schneider.

Thunberg & Carlström stated that C. O. Jensen in 1916 published a very precise account of sneezing sickness and clearly distinguished it from other similar diseases but was very guarded concerning aetiology. In 1933 he placed greater emphasis on infection. The Danish workers Gerhard Petersen and Axel Petersen in 1925 and 1926, respectively, considered the condition to be infectious, as did also Manninger and Eber & Mayn in 1934. Hovlund in 1937 stated that, as the theory of infection as the primary cause seemed to be unlikely, he was inclined

to suspect that a hereditary disposition was responsible for the disease. Thunberg & Carlström quoted Hovlund as saying later "The question of heredity or infection as primary cause is therefore an open one, but I have very emphatically maintained that infection is involved in any case, even if heredity should prove to be the primary cause".

Thunberg & Carlström, in the paper from which we have taken the foregoing information, gave a number of observations made on a farm of one of the authors and also described experiments carried out on farms. They considered that the disease gained entrance to Sweden from Denmark about 1930. Mention was made of cats and dogs, in contact with sick pigs, which developed symptoms of rhinitis and especially of purulent conjunctivitis. Incidentally the writer (R.G.) was unable to infect new-born kittens by nasal instillations of infectious nasal curettings. They also noted that as long as sows were nursing piglets having rhinitis they too showed symptoms of the disease but without the appearance of deformities. When the piglets were taken away, the symptoms disappeared in the sows. If such a sow were subsequently transferred to an uninfected house, her next litter would not as a rule be subject to the disease. They claimed that Thunberg had successfully taken advantage of this observation to stamp out the disease altogether without changing the breeding stock. As a result of their observations, these workers considered that they were entitled to consider the condition a purely infectious disease, in which inherited predisposition must play a very subordinate role.

Doyle *et al.* (3) in 1934 reported on a dystrophic rhinitis in Indiana. They pointed out the need to differentiate this condition from "bull nose", which is characterized by the presence of granulomatous swelling, with more or less necrosis in the central portion and in which the only distortion that occurs is due to the swelling *per se* or to destruction of bone.

THE SYNDROME

In general, the early symptoms are those of a cold, with occlusion of the nasal passages, watery discharge from the eyes and nose and not infrequently black, circular patches under the eyes due to the ocular discharge drying in this area and trapping dust. Sneezing is the most common symptom. Irritation of the nasal passages, as evidenced by the pigs vigorously rubbing their snouts on the ground or on any convenient object is highly suggestive. Difficulty in breathing is commonly observed and this may be very marked in unweaned piglets while feeding. Later, the discharge becomes thicker, collects in the nose, and is expelled by sneezing. Nasal haemorrhage, if present, is pathognomonic but it is not a constant symptom in field cases and seldom occurs in experimental infections. Development of the upper jaw is frequently interfered with, resulting in a deviation of the snout upwards or laterally. In these cases there is no incisor and poor molar contact, making it difficult for the animals to eat dry feed. A marked wrinkling of the skin of the snout is commonly observed.

Duthie (4) in 1947 pointed out that atrophic rhinitis was not a new disease in Alberta as he had reported it in the course of his work in 1934. At first it was thought that the poorly developed upper jaw and the prognathic lower jaw were always indicative of chronic rhinitis, but further investigation revealed that this malformation was present in nearly 90 per cent of young Yorkshire sows. This condition which was due to selective breeding for conformation, could easily be confused with rhinitis The shortening of the upper jaw resulted in the animals having no incisor contact, very poor molar apposition, and an upward tilt to the snout.

Arrest of growth has been suggested as the most serious result of the infection. Reports on this are variable and it may well be that secondary conditions are responsible for some cases but, in some herds at least, it is very definite. Not

much controlled evidence is available on this subject. Kristjansson & Gwatkin (5) found a difference between affected and non-affected pigs of 7·93 lb. at 56 days and 16 lb. at 168 days, to the disadvantage of the rhinitis-affected pigs when the figures had been corrected for difference in birth weights. In this study it was also shown that the infected pigs had lower birth weights. This may possibly have some aetiological significance. Certainly it suggests such a possibility. Mortality is low in uncomplicated rhinitis and is practically *nil* in experimental infections.

PATHOLOGY

Necropsy findings vary from slight shrinkage to complete decalcification of the superior and inferior turbinate bones and the ethmoid cells. There may be severe inflammation and large amounts of pus or the mucous membrane may appear quite clean. The sinuses may be affected and occasionally there is perforation of the bony plates. Secondary conditions may be present, such as pneumonia, but in experimental animals the changes are nearly always confined to the nasal passages. In field cases, allowance must be made for concurrent conditions and it is difficult to place proper values on these in connexion with the whole picture.

The gross changes in both natural and artificial infections may be quite striking if the infection has resulted in complete decalcification of the turbinal structures. Longitudinal section of the head and removal of the nasal septum reveals a large cavity in which the inferior turbinate is represented by a triangular strip of mucous membrane while nothing remains of the ethmoid but a collection of necrotic tissue and pus. In other cases, the changes may be confined to partial decalcification and deformity of the turbinates and no detectable changes in the ethmoid cells. The degree of destruction does not appear to bear any relationship to the infectivity of the nasal material.

Histopathological findings in primary cases are essentially negative. The nasal mucosa is usually normal with occasional round cell and polymorphonuclear infiltration. The destructive process appears to have a predilection for the submucosa. This coat is thin and few glands, mucous or serous, are present. No marked changes are seen in cartilage, bone or fibrous tissue.

METHODS OF INFECTION

Transmission of infection appears to be by contact and it is reasonable to assume, on the basis of experimental evidence, that the aerosols created by sneezing are taken in by the nasal route in the contact pigs. If part of a litter is infected by intranasal instillation of infective material, some of the non-infected animals will also become infected. In some experiments, more contacts than principals have been found infected at the end of the trials. The subcutaneous injection of highly infective nasal material, which produced complete and rapid decalcification of the turbinates when introduced by the nasal route, had no effect on the nares of litter-mates. Pigs do not develop lesions, even when infected by the nasal route, unless they are young. This no doubt was responsible for some of the negative results obtained in transmission experiments.

AETIOLOGY

The aetiology of the disease presents much of interest and reports on this have been very confusing. In view of the similarity of the symptoms and pathological changes, it is difficult to accept the concept that the causative agents vary in different countries. The possibility still remains that more than one agent may be involved but, in the event of this being true, it would seem reasonable to assume that there must be a common factor which, so far, has not been determined.

Virus

Filtrable agents have been suggested to be the cause of rhinitis but these reports have not been confirmed. In our hands and in the hands of others who have reported on this, filtrates have failed to produce rhinitis and have had no effect on chick embryos. Unfiltered nasal material cannot be used for egg work for obvious reasons, and treatment with penicillin and streptomycin destroyed the activity of the material for pigs, so that results with nasal scrapings were also negative in chick embryos.

Schofield & Jones (7) in 1947 failed to transmit the disease experimentally using filtered emulsions of mucous membrane and turbinate bones from cases of the disease.

Bacteria

In our early work a pasteurella was isolated by culture from 38 per cent of affected turbinates and 16 per cent of the non-affected ones. The majority of the latter group had been infected but had not developed lesions. For some unexplained reason, none of the cultures then tried produced rhinitis. In the early winter of 1952, clear-cut atrophic rhinitis developed in baby pigs following nasal instillations of a pure culture of *Pasteurella septica* (*multocida*), type B, fluorescent and mucoid. This culture was non-agglutinogenic but did produce a protective antiserum in pigs and rabbits, as demonstrated in mouse protection tests using 1000 M.L.D. of culture. The serum did not prevent the development of rhinitis in pigs by intraperitoneal injection or nasal instillation.

Subsequent experiments with cultures of *Past. septica* isolated from field cases of rhinitis also gave rise to rhinitis with clear-cut atrophy of the turbinated structures and litter-mates left in contact with the culture-infected animals also showed atrophy of the turbinates. Control animals were normal. Some of these strains were used by Dr. F. W. Schofield of the Ontario Veterinary College and he also produced atrophy of the turbinated structures in baby pigs by nasal instillations.

In 1952 Jones (8) noted that *Past. septica* was rarely absent from early lesions of rhinitis. McKay & Carter (9) in 1953 reported the production of rhinitis in pigs by nasal instillations of rabbit subcutaneous tissue which yielded only *Fusiformis* (*Spherophorus*) *necrophorus* and *Past. septica* on culture. They were unable to reproduce the condition with cultures of pasteurella alone.

Earlier attempts to infect laboratory animals at this Institute had proved negative, but further attempts with the nasal material from which the first successful strain had been isolated produced clear-cut rhinitis in rabbits and by rabbit passage of nasal material this has been carried for 15 passages over a period of 2 years. Cultures of *Past. septica* have produced rhinitis in rabbits but after the first or second passage the isolate fails to produce the condition.

Flatla & Braend (10) in 1953 reported that a species of *Pasteurella* seemed to be the most important aetiological factor in atrophic rhinitis. They produced typical atrophy of the turbinates with this organism. They did not consider the pasteurella organisms usually found in pigs to be the cause of the disease, but rather that there was a special type responsible for it. The controls in all experiments were normal.

Heddleston and his associates (18) recently reported on the examination of the nasal passages of pigs in two herds affected with rhinitis in Washington, D.C. They recovered *Past. septica* from 8 per cent of animals affected with rhinitis and 4·3 per cent of non-affected pigs. There were 17 weanlings, 144 slaughter animals and 11 adults in the 172 animals examined. It must be concluded from this that the majority of these animals had recovered from the infection but still showed the results, or that *Past. septica* is not the important aetiological agent in that district. In some cases we have found it necessary to resort to animal inoculation to demonstrate the organism but, in view of reports from other parts

of the U.S.A., it may be that some other aetiological or "trigger" organism is involved.

In another paper Braend & Flatla (11) discussed rhinitis in some detail. They were able to produce clear-cut atrophy of the turbinates with pasteurella and on one occasion produced a slight degree of atrophy with *Haemophilus suis* alone. They considered that predisposing factors are important, and they did not exclude the possibility that certain bacteria or viruses may be contributing factors in those cases where the disease is very infectious and associated with severe clinical symptoms.

Many organisms have been suggested as having an aetiological relationship to rhinitis, principally because they have been found to be present but, so far, the only organism that has produced clear-cut atrophy of the turbinates, or that has precipitated the condition, appears to be *Past. septica*. Among the bacteria suspected, *Corynebacterium pyogenes* and *Pseudomonas pyocyanea* (*aeruginosa*) have been prominently mentioned as playing an aetiological role in rhinitis but, in our hands and also at the Ontario Veterinary College, they had no effect on the turbinated structures. Messmore (12) considered *Erysipelothrix rhusiopathiae* as the probable cause but Borgman (13) demonstrated that this organism was not present in 4 rhinitis herds examined. He injected pigeons with nasal material, which had no effect on these birds, and later proved that they were susceptible to injections of *E. rhusiopathiae*.

Pleuropneumonia-Like Organisms

In 1953, Switzer (17) reported the isolation of a filtrable agent by the use of a Selas O2 candle. The filtrate contained fine bodies, which from work at this Institute appeared to be pleuropneumonia-like organisms (PPLO). Selas filtrates in our hands, and in the hands of others, did not produce rhinitis. Cultures of PPLO isolated from cases of rhinitis at this Institute had a slight effect on the nasal passages in one instance but this was very indefinite and all the other animals were negative, except one from which *Past. septica* was isolated.

Trichomonads

Switzer (14) suggested an aetiological role for trichomonads. Experiments at our Laboratory showed them to be present in some infected and some non-infected animals but they could not be demonstrated in frozen material which was still infective for baby pigs. Spindler *et al.* (15) in 1953 produced rhinitis with nasal material containing trichomonads, but these organisms were in nasal material and not free from other possible aetiological agents. Levine and his associates (16) in 1954 were unable to produce rhinitis with bacteria-free trichomonads.

DIAGNOSIS

The situation in regard to diagnosis is extremely unsatisfactory. Certainly a herd diagnosis can be made clinically in most cases but it is not possible to pick out all the infected animals. Changes in many cases are only detected at necropsy and work at the Animal Diseases Research Institute, Hull, Quebec, has shown that a sow may be capable of infecting her litter and yet show no changes herself at necropsy. Illumination of the nose has been tried (6) with a fair degree of success, but this method does not reveal slightly affected animals and could not possibly reveal the carrier animal which has no lesions. Blood tests and skin tests with cultures, filtrates and nasal material have failed to differentiate infected and normal animals. X-ray methods have also been tried by some workers but without very much success.

TREATMENT

It is probably a fair statement to say that treatment and control have proved disappointing, which is not surprising in view of the indefinite knowledge on the aetiology of the condition. Under experimental conditions, the instillation of 100 mg.

of streptomycin daily for several days was successful in most instances in preventing the development of lesions which occurred in untreated controls in both natural and artificial infections. In the former group, all piglets were suckled by the sows. In the latter, the treated animals were isolated at the commencement of treatment. Intraperitoneal injections and nasal instillations of *Past. septica* antiserum did not prevent the development of lesions in most cases. Jones (8) tried *Corynebacterium pyogenes* antiserum and convalescent serum without any prophylactic effect in natural infection. A toxoid of the organism was also tried following an initial injection of hyperimmune serum. He did not obtain any protection in unweaned piglets in infected herds with one injection of pasteurella antiserum followed by two doses of killed culture vaccine. Our experience with pasteurella serum and killed culture vaccines in prevention of artificial infection was the same. He found that an intramuscular injection of 100,000 μg. of streptomycin, given three times during the first month after birth reduced the incidence of rhinitis by a significant degree but did not control the disease completely.

CONTROL

In 1952, the State of Kansas (19) issued regulations governing atrophic rhinitis in swine. It was recognized as an infectious and contagious disease to be reported to the State Livestock Sanitary Commissioner when diagnosed. Infected herds were to be quarantined until official release was obtained and during this time animals could be sold for slaughter only. After disposal of all pigs, and cleaning and disinfection, a period of 3 months was required before restocking. It was also made unlawful to import swine into the State without an official health certificate.

The Ministry of Agriculture and Fisheries circulated a notice in May 1924 (20) that, for the first time on record, atrophic rhinitis had been confirmed in pigs in Great Britain. Infection appeared to have been introduced by a Landrace pig imported into England from Sweden. The Minister of Agriculture then made the Atrophic Rhinitis Order, 1954, which deals with the definition of the disease, the detention and isolation of swine and precautions to be adopted to prevent the spread of infection, and cleansing and disinfection. A second order dealt with compensation to be paid to owners of slaughtered animals. Where the slaughtered animal is affected with rhinitis, one half of the value of the animal immediately before it became so affected is paid. In other cases, the value of the animal immediately before it is slaughtered is paid. An article (21) was published in June 1954 drawing attention to the condition and outlining the historical aspects, systems, pathology, inherited susceptibility and attempts at transmission.

CONCLUSIONS

In conclusion, it can be said that present knowledge on atrophic rhinitis is in a confused state. There is no agreement on the aetiology and, consequently, efforts to develop a method of diagnosis are hampered. For the same reason, methods of treatment and control are empirical and, as far as reported, not very satisfactory. It may be that several organisms are capable of producing rhinitis, but no published work to date indicates that the condition has been reproduced in a clear-cut manner by any pure culture except *Past. septica*. In spite of this, however, it can only be stated that this organism has produced atrophic rhinitis but not that it is the cause of atrophic rhinitis.

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The Epidemiological Role of Animal Reservoirs in Human Leishmaniasis and Trypanosomiasis

BY

CECIL A. HOARE, D.Sc., F.R.S.

Wellcome Laboratories of Tropical Medicine, London.

It is well known that among the infectious diseases affecting man there are a number in which lower mammals play an important epidemiological role as reservoirs of the infection. Such infections, which are common to man and other animals, are known as zoonoses, and their study provides a field where veterinary and medical sciences meet on common ground, especially in the sphere of epidemiology.

Among the zoonoses caused by bacteria and viruses the best known are plague, tick-borne relapsing fever, brucellosis, seasonal encephalitides, yellow fever and tularaemia. Protozoal infections of this type are represented by balantidiosis, toxoplasmosis, leishmaniasis, trypanosomiasis, and possibly amoebiasis. The subject of zoonoses is essentially a chapter in the natural history of infectious diseases, which it is proposed to illustrate by leishmaniasis and trypanosomiasis.

LEISHMANIASIS

Both the cutaneous and visceral types of leishmaniasis, known as Oriental Sore and Kala-Azar respectively, provide examples of diseases in which the human and animal hosts are closely connected epidemiologically.

Oriental Sore. The epidemiology of cutaneous leishmaniasis has been studied chiefly in Soviet Middle Asia. Here it should be mentioned that Russian workers (Kojevnikov, 1941, 1942*b*; Latyshev and Kriukova, 1942) have established the existence in that region of two distinct nosological types of oriental sore. One is the so-called "dry" or urban type, which is prevalent in towns and is characterized by a chronic course with late ulceration; the other is known as the "moist" or rural type: it occurs in the open country and runs an acute course, with early ulceration. Kojevnikov (1942*a*) has shown that these two variants are also immunologically distinct. According to observations of the Russian workers, the incidence of infection of human beings with the "moist" type of oriental sore is particularly high in isolated rural settlements adjoining the open desert. It was also noted that people frequently became infected with *Leishmania tropica* after visiting uninhabited desert areas. Subsequently it was demonstrated that in these cases the disease was acquired from infected sandflies (*Phlebotomus*) living in the burrows of ground squirrels, especially the gerbil (*Rhombomys opimus*). Moreover, these rodents also had typical sores on their ears and noses, from which *L. tropica* was recovered. Finally, it was proved experimentally that the gerbil parasites were infective to man and *vice versa*. It was thus demonstrated that these rodents represented reservoir hosts of human cutaneous leishmaniasis of the "moist" type (*cf.* Hoare, 1944).

In the light of these observations, an attempt was made to control the disease in a primitive settlement situated in a desert area of Turkmenistan, where 70% of

the population were affected with oriental sore of the "moist" type. After having ascertained that sandflies do not fly beyond 1,500 metres from their breeding places in the desert, some half a million gerbil burrows were poisoned with chloropicrin over an area within a radius of 1,200 m. from the village. A year later it was found that the incidence of human infection in this village had dropped almost to zero.

This campaign not only successfully eradicated a hyperendemic focus of cutaneous leishmaniasis, but it also proved the correctness of the epidemiological premises upon which the experiment was based.

The position in large rural settlements of Soviet Middle Asia is somewhat different, for here agricultural development had forced the wild rodents to retreat into open country, leaving the oasis practically free from infection, except along the periphery adjoining the open desert, where the incidence of human infection is low (2–3%) (Latyshev *et al.*, 1951). A further step in the evolution of the epidemiology of oriental sore is found in the towns of Middle Asia, where the "moist" type of the disease is replaced by the "dry" urban type. It would appear that in urban areas the sandflies had completely lost contact with their wild rodent hosts, and established themselves in the proximity of man, having become synanthropic or "domiciliated". They presumably now transmit cutaneous leishmaniasis directly from man to man, since in towns no reservoir mammalian hosts have hitherto been demonstrated with certainty (Hoare, 1944).

In addition to the Soviet Union, the epidemiology of oriental sore was studied in Iran, where Ansari & Faghih (1953) likewise traced the source of human infection with the "moist" type, in the desert area of Sarakh, to infected gerbils, *R. opimus*. There is also circumstantial evidence of the existence of reservoir hosts of human cutaneous leishmaniasis in Africa. Thus, "bouton de Biskra" in North Africa has all the characteristics of the "moist" type of oriental sore (Latyshev *et al.*, 1953), and this type of the disease has also been recorded from French West Africa (Lefrou, 1948). Moreover, gerbils and ground squirrels in North Africa have been successfully infected with human strains of *L. tropica*. In this connexion it is significant that the ecology of these African desert rodents is like that of the rodents which are proven carriers of oriental sore in Asia (Hoare, 1944). Finally, it has recently been suggested by del Ponte (1952) that the agouti (*Dasyprocta aguti*) is the reservoir host of "Espundia" (*Leishmania braziliensis*) in the forest region of Paraguay.

Kala-Azar. Visceral leishmaniasis is represented by three main types of disease: (a) the classical kala-azar confined to India, which affects chiefly adult people, but does not occur in dogs; (b) the Mediterranean or infantile kala-azar, which is prevalent in children and occurs throughout the Mediterranean Basin, in Middle Asia, China, and Central and South America; (c) Sudanese kala-azar, which chiefly affects the same age-groups as in India and likewise does not occur in dogs.

In all the areas affected by Mediterranean kala-azar, dogs are found to be naturally infected with *L. donovani*, and there is intimate connexion between the human and canine diseases. In fact, there is also a close parallelism in their geographical distribution and incidence. Moreover, it has been demonstrated—in Sicily, Middle Asia and China—that systematic destruction of dogs in endemic areas has brought about a marked diminution in the incidence of human infection.

The dog is therefore the reservoir of the Mediterranean form of visceral leishmaniasis, and the disease is in fact a zoonosis, being prevalent in up to 20% of these animals, from which it may spread to children. Transmission of the infection

to man is facilitated by the fact that in dogs the parasites invade the skin, whence they are readily picked up by the sandfly-vector (Adler & Theodor, 1932).

Nothing was known about reservoir hosts of visceral leishmaniasis among wild mammals until recently, when Latyshev *et al.* (1951) investigated outbreaks of kala-azar in Middle Asia, among workmen engaged in the reclamation of previously uninhabited land in Tadjikistan. The epidemiological situation pointed to the existence of some natural local focus of the infection, which was confirmed by the discovery of spontaneous visceral leishmaniasis among jackals, *Canis* (*Thos*) *aureus*, which are common in this region. It was also established that natural enzootic foci of the disease had always been present in the area in question, and when human beings first came in contact with jackals, they acquired the infection from sandflies feeding on these animals, which thus became reservoir hosts. Subsequently, when the primitive settlements developed into modern villages surrounded by cultivated land, the jackals were forced out or eliminated.

In some instances this caused the epidemiological chain to rupture, bringing the outbreaks of the disease to an end. However, in other areas, represented by towns of Middle Asia (e.g. Tashkent, Samarkand, Bokhara) and the Caucasus (e.g. Erevan, Tiflis), where kala-azar is endemic, the disease had adapted itself to man as one of its hosts, while retaining its infectivity for domestic dogs, whose ancestors must have originally acquired the infection from their wild relatives, the jackals. It is conceivable that in the Mediterranean area dogs might similarly have acquired their infection, in the remote past, from some wild Canidae, *e.g.* foxes. In fact, the role of foxes as reservoir hosts of kala-azar in Kirgizia (Middle Asia) is already suspected (Latyshev *et al.*, 1951), while Deane & Deane (1954 *a,b*) have recently reported that in Brazil foxes (*Lycalopex vetulus*), captured in the vicinity of a town where kala-azar is endemic, were naturally infected with *L. donovani*, but in the town itself the reservoir hosts were represented by dogs. Finally, Heisch (1954) provided circumstantial evidence of the existence of animal reservoirs of human kala-azar in Kenya, where the disease is of the Sudanese type.

The situation in India is still obscure, for all attempts to discover a reservoir host have so far failed. However it is difficult to admit that the epidemiology of Indian kala-azar is different from that in other types of leishmaniasis: it is conceivable that in India infection with *L. donovani* also occurs in some lower mammal—other than the dog—but remains undetected (Hoare, 1945, 1954).

TRYPANOSOMIASIS

We now turn to the epidemiology of human trypanosomiasis, represented by Sleeping Sickness in tropical Africa and Chagas' disease in Central and South America, to which may be added infections with *Trypanosoma rangeli* in some parts of South America.

Sleeping Sickness. As is known, Sleeping Sickness is represented by two forms of the disease: the chronic Gambian type and the acute Rhodesian type, but the causative organisms of both are closely related or even identical, *T.rhodesiense* probably being a virulent race of *T. gambiense*. Moreover, both these species are morphologically indistinguishable from *T. brucei*, which causes nagana in livestock.

The question regarding the reservoir hosts of sleeping sickness has not been fully elucidated (*cf.* Hoare, 1948), mainly because in the endemic areas tsetse-flies, on the one hand, and domestic or wild mammals, on the other, may be naturally infected with any of these trypanosomes, whose nature cannot always be established with

certainty. Therefore, in most cases, the identification of the trypanosomes detected in natural infections is based on epidemiological data. Thus, trypanosomes found in animals from localities where the human disease is unknown are most probably *T. brucei,* while those occurring in areas where the Rhodesian disease is endemic might be either *T. brucei* or *T. rhodesiense,* their differential diagnosis depending upon the results of inoculation of human volunteers. In endemic areas of the Gambian disease *T. brucei* can be differentiated from *T. gambiense* by its virulence for laboratory rodents.

Since both *T. rhodesiense* and *T. gambiense* have also been maintained by cyclical transmission through wild and domestic ungulates for several years, without losing their infectivity to man, it is concluded that certain mammals might constitute a source of human infection. The relative importance of the human and animal reservoirs differs according to the type and distribution of the disease (Buxton, 1955). Thus, it is thought that the Rhodesian disease, which is usually encountered on the fringes of thinly populated bush-country abounding in big game, is transmitted by game tsetse (*Glossina morsitans* group) from antelopes to man and from man back to antelopes, the latter representing important reservoir hosts even in localities which had been depopulated for some years (Fairbairn, 1948). However, in the Gambian disease, which usually occurs in relatively well-populated localities with a scanty wild mammalian fauna, man appears to be the only essential host, deriving his infection from waterside tsetse (*G. palpalis* group). However, there is both experimental and epidemiological evidence that some domestic animals, especially pigs and goats, living in close association with man in endemic areas might serve as reservoir hosts of human infection (Duke, 1928; Hoof, 1947).

Chagas' Disease. In the case of Chagas' disease the role of lower mammals as sources of human trypanosomiasis is much clearer than in sleeping sickness, for in the New World numerous wild animals are naturally infected with *T. cruzi,* which is transmitted by triatomid bugs living in association with them. Infected bugs are encountered throughout the Americas, not only in localities where the human disease is endemic but also outside these areas, where they inhabit the burrows and nests of wild mammals such as armadillos (*Dasypus*) and opossums (*Didelphys*), which are the chief reservoir hosts of Chagas' disease. In such natural foci human beings may become infected with *T. cruzi* accidentally, when attacked by "wild" bugs outdoors (Dias & Chandler, 1949).

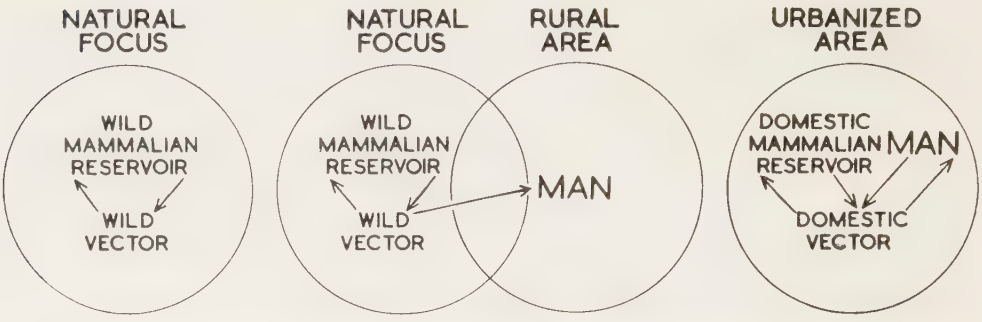
However, the main source of human infection are synanthropic triatomid bugs which had established themselves in human dwellings and then became "domiciliated". In such cases the role of reservoir hosts is taken over by domesticated mammals, the most important of which are dogs, cats, and probably pigs as well, all of which have been found naturally infected with *T. cruzi.* It is interesting to note that Chagas' disease has not been recorded in the U.S.A., in spite of the fact that in the southern States (Texas, New Mexico, Arizona and California) wood-rats (*Neotoma*) and the triatomid bugs living in their nests are naturally infected with *T. cruzi,* capable of infecting man under experimental conditions (Packchanian, 1943).

Trypanosoma rangeli. Finally it may be mentioned that in parts of South and Central America (Venezuela, Colombia, Guatemala) human beings and dogs are commonly infected with a trypanosome, *T. rangeli* (=*T. ariarii*) which causes no symptoms of disease in either of these hosts. Trypanosomes indistinguishable from this species have also been recorded from *Cebus* monkeys and opossums (*Didelphys*) which are regarded as reservoir hosts of the human and canine infection (Hoare, 1953; Pifano, 1954; Floch & Fauran, 1954; Zeledon, 1954). *T. rangeli* is transmitted by the triatomid bug, *Rhodnius prolixus.*

DISCUSSION

From the account given above, it is evident that all the various forms of human leishmaniasis and trypanosomiasis have a common epidemiological pattern, which is illustrated in the accompanying diagram.

Figure One



Epidemiological patterns in leishmanial and trypanosome infections of man, showing role of animal reservoirs in various enzootic and endemic foci (circles): arrows indicate circulation of infection from mammalian donor—through vector —to recipient.

It is generally found that, in addition to endemic foci of human infection, these diseases have natural enzootic foci situated in the open country, where the infection circulates by insect-transmission from donor to recipient hosts, represented by wild mammals. One of the characteristics of these natural foci is their complete independence of man and his domestic animals. However, when human beings temporarily enter such an enzootic locality, or settle under primitive conditions in its vicinity, they expose themselves to attacks of the wild vectors and become infected. With time, agriculture and general economic development lead to an increase in the size and to urbanization of the human settlements, bringing about various changes in the epidemiological situation. Thus, (1) contact between man and wild nature, with its reservoir hosts and vectors, may be broken, with the result that human infection disappears from the settlement; or (2) the wild vectors may establish themselves within the settlement and become synanthropic or "domiciliated" there, transmitting the infection directly from man to man (*e.g.* in urban oriental sore, Indian kala-azar, Gambian sleeping sickness); while (3) in other cases domestic mammals may replace the wild animals as reservoir hosts (*e.g.* Mediterranean kala-azar, Chagas' disease). The epidemiology and host-parasite relations in human leishmaniases and trypanosomiases thus provide clues to the genesis of endemic foci by evolution from purely enzootic foci, the course of which—in the case of leishmaniasis—has been analysed by Kriukova & Latyshev (1948). Here it should be mentioned that the wild reservoirs are typically symptomless carriers of the infection. This fact points to a stable adaptation between host and parasite, which reflects the antiquity of their association in primitive enzootic foci, as compared with that in more recently evolved endemic foci. These human diseases should therefore be regarded as zoonoses, with reservoir hosts represented by either wild or domestic mammals, or both. The relation of the two kinds of reservoir hosts to human diseases caused by haemoflagellates in different epidemiological situations is summarized in the accompanying table.

TABLE 1.

Animal Reservoirs in Human Leishmaniasis and Trypanosomiasis

Diseases	*Vectors*	*Reservoir Hosts in:*	
		Natural Focus & Rural Area	*Urbanized Area*
Leishmaniasis:			
Cutaneous, moist type	Sandflies	Gerbils, ground squirrels	None
Cutaneous, dry type	„	None	?
Cutaneous, Espundia	„	? Agouti	None
Visceral, Mediterranean type	„	Jackals, Foxes	Dogs
Visceral, Indian type	„	?	?
Trypanosomiasis:			
Rhodesian Sleeping Sickness	Tsetse-flies	Antelopes	None
Gambian Sleeping Sickness	„	None	(? Pigs, Goats)
Chagas' disease	Triatomid bugs	Armadillos, Opossums	Dogs, Cats, Pigs
Trypanosoma rangeli	„ „	*Cebus* monkeys, Opossums	Dogs

Finally the existence of natural enzootic foci of the human diseases in question (as well as in other zoonoses) has certain practical implications. Such foci may remain undetected and, as it were, dormant for indefinite periods of time, in fact as long as human beings do not come in contact with them. They therefore constitute a potential epidemiological danger, and it is important that their existence and localization should be recognized beforehand. Since such foci or biotopes are characterized by definite ecological peculiarities—determined by topography, climate, vegetation and other environmental factors—these can serve as indicators of the presence of certain diseases in such areas. A knowledge of what Pavlovsky (1948) calls "the landscape epidemiology" is therefore useful in determining the potential danger of unknown territories into which man is about to penetrate, *e.g.* in the course of reclamation work, during military operations or movements of troops, and when camping in such places. Thus, in desert areas inhabited by burrowing rodents the presence of oriental sore might be suspected, while in tropical Africa, bush country with big game or river banks with thick vegetation might indicate the presence of sleeping sickness. If the potential danger of such localities is recognized, they can either be avoided, or appropriate measures can be taken to protect human immigrants from infection.

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Nutrition and Fertility in the Bull

BY

D. E. Tribe, B.Sc. (Agr.), Ph.D. and R. B. Cumming,* B.V.Sc.

School of Veterinary Science, University of Bristol.

It is a common impression that the last decade has seen a serious increase in bovine infertility. [A number of reviews have dealt with various aspects of this general theme (*e.g.* Bartlett, 1949; Asdell, 1949, 1952, 1953; Gilmore, 1949; Sykes, 1949; Hignett, 1950; Young, 1953).] Although it is held by some that nutrition often plays a critical role in bovine infertility, (Hignett, 1950, 1952) it is more generally accepted at the present time that only in a small proportion of field cases is there a nutritional cause. These cases are almost always due to general underfeeding, so that deficiencies are multiple rather than specific, and before reproductive efficiency is reduced an animal usually shows other signs of general malnutrition (Asdell, 1949, 1953). The concern of most workers, however, has been with the female and except for the general review by Reid (1949) little attention has been paid to the possible relationship between nutrition and infertility in the male.

The present review discusses firstly the possible effects on male fertility of specific dietary deficiencies, then considers the plane of nutrition in relation to sexual function and finally indicates how, in practice, these requirements may be satisfied. It can be argued that any nutritional imbalance may, by reducing an animal's general health, impair its fertility. Such non-specific considerations are, however, beyond the scope of this review.

VITAMINS

Fat-Soluble Vitamins

Vitamin A. It has been demonstrated by many workers that a lack of vitamin A in the diet of bulls delays their sexual maturity, suppresses sexual interest, and interferes with spermatogenesis. For example Hodgson *et al.* (1946) produced typical symptoms of avitaminosis A in 12 dairy bulls. Nevertheless, although the bulls were in an advanced state of deficiency, semen collected was used successfully to serve cows and quite normal calves were born. The semen from such bulls was of low spermatozoan concentration, had a high percentage of abnormal spermatozoa, a high pH, and did not store well. An inability to mount and deliver semen was characteristic, but was thought to be the consequence of general inanition and weakness, rather than a specific symptom of avitaminosis A. From the point of view of the feeding of bulls at artificial insemination centres it is important that these workers also found that if sufficient vitamin A is fed during growth up to the age when breeding starts bulls will continue to produce fertile semen for long periods although the dietary source of vitamin A is withdrawn. After the feeding of supplemental carotene to deficient bulls a dramatic improvement in their condition occurred and they could then be used for breeding. However, if marked deficiency symptoms appeared before the onset of puberty, the bulls failed to breed. The epithelium of the seminiferous tubules had degenerated and the addition of vitamin A to the diet did not remedy this damage. These findings are in substantial agreement with those of other workers (Guilbert & Hart, 1935; Sutton *et al.*, 1940; Madsen *et al.*, 1942; and of Erb *et al.*, 1944, 1947) and emphasize that under normal field conditions avitaminosis A is unlikely to influence fertility since before it does so other obvious and general symptoms will be seen and the diet improved. In addition

*Present address: *P.O. Box* 1, *Sandown, Johannesburg, South Africa*

Jones *et al.* (1946), working with Jersey bulls have shown that daily carotene levels of from 15 to 35 μg. per kg. body weight are sufficient to satisfy requirements, which are no greater for normal reproduction than they are for normal growth (Jones *et al.*, 1942). This means that if 35 μg./kg. is taken as the standard requirement, a 2,000 lb. bull will need 30 mg. carotene per day. Fresh grass contains from 30 to 60 mg. carotene per 100 g. dry matter and similarly molassed grass silage contains from 20 to 50 mg. (Woodman, 1948). In other words two pounds of grass or silage per day provide more than enough carotene for a large mature bull. These calculated requirements may be considered too low on the ground that they do not allow for sufficient storage of vitamin A in case of emergency. However, as bulls are usually allowed generous access to green food there seems little likelihood, at least in areas of adequate rainfall, of such a deficiency being responsible for a decline in fertility.

Vitamin E. On the basis of the early investigations into the importance of vitamin E in the nutrition of the rat (Evans *et al.*, 1927; Mason, 1933) the name 'anti-sterility vitamin' was sometimes used to describe its activity and importance. However, subsequent work showed that there was a large species variation in response to vitamin E and as far as farm animals are concerned the description is a misnomer. With farm herbivora no abnormality of reproductive behaviour has yet been shown to follow vitamin E deficiency although the treatment of infertile farm animals with vitamin E or wheat germ oil has led to extravagant claims for the therapeutic value of the vitamin.

In a number of exact experiments Gullickson and his associates fed bulls rations composed of foods which were low in their vitamin E content and which had been heated at 100°C. for long periods (Gullickson *et al.*, 1944, 1949; Gullickson, 1949). These rations were not capable of supporting reproduction in rats and yet bulls, even after some years on the rations, had normally developed reproductive organs and produced spermatozoa which were normal in quantity, activity, morphology, longevity, and fertilizing capacity. Similar critical work on sheep (Thomas *et al.*, 1946) and goats (1938) indicates that for normal reproduction these species do not require vitamin E. On the other hand there are numerous reports or the efficacy of wheat germ oil in the treatment of sterility in cattle and horses by Vogt-Møller and others (Vogt-Møller & Bay, 1931; Moussu, 1935; Vogt-Møller, 1939; Way, 1942). These have been reviewed by Blaxter & Brown (1952), who summed them up by writing:

> "They are to be regarded not merely as inconclusive but as valueless in establishing that there is any need for the vitamin, either as a nutritional entity or as a pharmacological adjuvant".

Russian workers have claimed marked improvement of fertility and libido in bulls when wheat germ oil was given (Timin & Pereturina, 1941). Experiments by Salisbury (1944) however, failed to demonstrate any effect of wheat germ oil on the fertility of bulls kept for more than a year in the exacting conditions of an insemination centre.

Vitamin D. There is no direct evidence to suggest that vitamin D is of special importance in the nutrition of the male. However, as will be mentioned later, both an excess of calcium and a deficiency of phosphorus are alleged to produce infertility, and it has been argued (Abrams, 1951) that these minerals have been incriminated in circumstances in which it would have been at least as reasonable to blame avitaminosis D.

Water-Soluble Vitamins

Vitamin C. Of the water-soluble vitamins the one most likely to be concerned with reproductive performance is vitamin C but the evidence concerning this vitamin

is confused and contradictory and it is therefore difficult to make a precise evaluation of its importance.

On the one hand the *prima facie* case for linking vitamin C with reproduction is based chiefly upon the observations that the ascorbic acid levels are high in a number of tissues concerned with reproduction (Mason, 1939) and that vitamin C influences, or is influenced by, a number of reproductive hormones (Di Ció & Schteingart, 1942). In opposition to this it is well-known that of all species, only human beings, monkeys and guinea pigs exhibit the symptoms of scurvy as a result of a deficiency of ascorbic acid and in this condition there is no specific reproductive disturbance (Mason, 1939). Male guinea pigs showing scurvy possess quite normal testicular tissue and spermatozoa (Friedman & Turner, 1939). Although one report has suggested that under certain conditions cattle may develop scurvy (Duncan *et al.*, 1944) it is now well established that farm stock can normally be relied upon to synthesize their own requirements of vitamin C (see, *e.g.* Wallis, 1943).

It has been claimed (Phillips *et al.*, 1941) that the ascorbic acid content of semen was much lower in bulls of poor fertility (2 mg. per cent) than in bulls of high fertility (3–8 mg. per cent). In 1940 the same authors had reported that 80 per cent of bulls with histories of low potency, slow breeding and general sex indifference, improved after the subcutaneous injection of ascorbic acid. Scheidenhelm *et al.* (1942) fed chloretone to three bulls of poor fertility as a method of increasing the ascorbic acid level of the blood and reported that their fertility improved markedly. These claims have not been supported by other workers and more recently Reid *et al.* (1947) showed that the semen of some bulls contains large amounts of sulphydryl substances which are measured as ascorbic acid when the analytical method employed by Phillips is used. As Reid (1949) has pointed out this finding discounts the physiological basis for ascorbic acid therapy in bulls.

In the face of this evidence it is fair to conclude that vitamin C is probably not of importance so far as male fertility is concerned.

Vitamin B Complex. Moore & Samuels (1931) demonstrated that a vitamin B complex deficient diet fed to male rats produced regressive changes in the accessory reproductive organs and that this could be counteracted by the administration of testicular hormone or anterior pituitary extracts. They therefore concluded that the primary lesion was located in the pituitary gland, and that as a result of the diminished hypophyseal activity the testes received insufficient gonadotrophic stimulus and thus were unable to produce the male sex hormone required for the normal function of the accessory glands. This work has been confirmed by Mulinos & Pomerantz (1941) and by Grayhack & Scott (1952), and Koller (1946), Dunn *et al.* (1947) and Elson & Koller (1948) also showed that spermatogenesis in laboratory animals may be affected by a vitamin B deficient diet. In the bull however, there is little danger of such deficiencies since the requirements are normally satisfied by the bacterial synthesis of these factors in the rumen. The possibility remains that the ingestion of anti-vitamin factors (*e.g.* the anti-thiamine present in bracken) may induce a deficiency of one or more of the components of the B-complex. Even in the event of such an unlikely contingency, however, other more obvious symptoms will probably appear before reproductive efficiency is lowered.

MINERALS

Since the studies of Theiler and his associates in South Africa (*e.g.* Theiler *et al.*, 1928; Theiler & Green, 1932) a great deal of attention has been paid to the importance of phosphorus in maintaining efficient reproduction in cows and heifers. There is now no doubt that uncomplicated aphosphorosis will impair a female animal's breeding capacity, but it is probable that very few cases in the field are of

an uncomplicated nature (*e.g.* most phosphorus deficient foods are also deficient in protein). It is not however, within the scope of this review to discuss this complicated subject since no reports (except that of Hignett, 1950, mentioned below) appear to have outlined the effects of phosphorus deficiency on the reproductive capacity of the bull.

There is little evidence to suggest that calcium is of special importance in the feeding of the male. It has been claimed by Hignett (1950) that an excess of calcium (or alternatively a wide Ca: P ratio) may lead to infertility in bulls. In an outline of his findings, without detailed presentation of experimental evidence, he maintains that an increase of dietary calcium would depress semen quality, as would a widening of the Ca: P ratio. Certain of his bulls were particularly susceptible to changes in the calcium intake in the ration, and a young bull for a prolonged period on a diet with a high calcium/low phosphorus ratio suffered an irreversible testicular degeneration leading to sterility. However, since no further reports on this subject have yet appeared it is impossible to assess its practical importance.

There is a possibility that manganese may influence the fertility of bulls. Certainly a manganese deficient diet causes a degeneration of the testicular epithelium of the male rat (Boyer *et al.*, 1942) and of the male rabbit (Smith *et al.*, 1944). Orent & McCollum (1931) suggested that this effect on testicular tissue was direct, rather than indirect through the pituitary gland, since a deficiency does not influence the oestrous cycle in females.

Although little is known concerning manganese deficiencies in ruminants (see *e.g.* Bentley & Phillips, 1951) it has been suggested (Lardy *et al.*, 1942) that supplementation of a low manganese ration with this element improves the poor quality semen and the lowered fertility of some bulls.

Deficiency or excess of several other minerals and trace elements have at one time or another been suggested as causes of infertility. There have, for example, been reports of infertility in cattle due to iodine deficiency. Spielman *et al.* (1943) performed thyroidectomy on one bull and reported that although semen production continued for 453 days the bull was sexually disinterested. Thyroid therapy led to the restoration of normal breeding behaviour. Hignett (1952) suggested that under field conditions iodine deficiency may lead to loss of libido and a deterioration in semen quality but this claim has never been substantiated. An excess of molybdenum has been shown to produce, in addition to the well known symptoms of molybdenosis, a lack of sexual interest in bulls coupled with degeneration of the germinal epithelium and little or no spermatogenesis (Thomas & Moss, 1951). A deficiency of copper is known to delay the onset of puberty in the female but, as with cobalt and zinc deficiencies, there is no evidence to suggest that a deficiency of the element has a specific influence on the reproductive capacity of the male.

Undoubtedly the importance of the nutrient deficiencies and excesses so far described is usually very limited, for such specific conditions occur only rarely. If malnutrition should be responsible for a decline in fertility then it is in all probability of the multiple deficiency type in which protein and even energy restrictions are the main factors in a generally inadequate ration.

PROTEINS

In view of the microbial synthesis which normally occurs in the rumen it is more than usually dangerous to draw theoretical analogies and practical conclusions for the feeding of ruminants from the many experiments which have been reported in which laboratory animals have been maintained on various quantities and qualities of protein. While this work reflects the general importance of the total amount of protein and of the part played by amino-acids in the maintenance of fertility, it is reasonable to assume that the same detailed attention to protein dietary constituents is unnecessary when constructing rations for bulls. As far as the quantity of protein is

concerned Branton *et al.* (1947) showed that a concentrate mixture containing 12 per cent total protein together with mixed hay (9 per cent protein) supplied sufficient protein for the maintenance of body weight as well as satisfactory semen production. They considered this to be a perfectly adequate diet for use in A.I. centres although bulls fed a concentrate mixture with a total protein content of 20 per cent produced a slightly greater concentration of spermatozoa, a lower ejaculate volume and motility, and less spermatozoa per ejaculation than did bulls receiving lower protein feeds. The results of Larsen *et al.* (1944) also encourage the belief that a high intake of digestible protein increases the fertilizing capacity of bulls, although there is evidence from New Zealand (Webster, 1932) that sterility in grazing bulls may be associated with an unusually high crude protein content of the dry matter of the grass.

The work that has been done on the quality of proteins for bull feeding is very contradictory. Reports from Russia (Smirnov-Ugrjumov, 1937; Smirnov-Ugrjumov & Laptev, 1938; Milovanov & Sokolovskaya, 1948) emphasize that animal protein should be an essential constituent of a stud bull's diet, for such foods, particularly blood meal and meat and bone meal, increase sexual activity and improve the quality of the semen. In the absence of any evidence to the contrary, the practice of including animal protein in the diets for bulls in British A.I. Centres is now usual. However, more recently Branton *et al.* (1949) have shown that under American conditions, animal proteins have no special advantage over vegetable proteins and it seems justifiable to conclude from this study together with the results of Reid *et al.* (1948) and of Quicke *et al.* (1950) that, provided sufficient energy and protein is supplied in the ration the source of the protein is of no particular importance. Since animal proteins are normally in short supply, and therefore expensive, it may be a considerable economy if they can be replaced in bull rations by plant proteins.

There is some evidence to suggest that the feeding of synthetic thyroproteins to bulls may increase the quality of their semen and their fertility (Schultze & Davis, 1946). However, much information on the feeding of thyroproteins to ruminants has now accumulated (see review by Blaxter, 1952) and in view of their possible harmful effects after prolonged administration their inclusion in the rations of stud bulls is not to be recommended.

CARBOHYDRATES AND FATS

Except as a general source of energy (in which capacity they may be replaced by fats and proteins) the carbohydrates do not appear to be of special importance as far as reproduction is concerned. Certainly a diet containing no carbohydrates had no effect on the regularity or frequency of oestrous cycles in female rats (Evans & Bishop, 1922). However, the feeding of a diet in which 20 per cent of starch was replaced by sucrose resulted in a precocious sexual development and subsequent sterility in rats (Whitnah & Bogart, 1936). A physiological explanation of this observation has not been offered. Both of these observations were made on the female only and there is no published evidence which relates to the male. However, Hignett (1950) has stated, on the basis of his own unpublished work on bulls, that "it did appear that starch intake tended to influence semen quality to a greater extent than did protein". In the absence of further evidence it is impossible to dogmatize on this question but a physiological explanation of this observation is hard to find.

Deficiencies of essential fatty acids are known to have an adverse influence on the reproductive efficiency of rats (see, *e.g.* Burr & Burr, 1930; Maeder, 1938). In the case of ruminants, however, it is extremely unlikely that such deficiencies ever take place and their possible effects have never been investigated.

PLANE OF NUTRITION

It is difficult to investigate the effect on sexual function of the plane of nutrition since it is nearly impossible to produce large quantitative differences in the nutritional level without also involving qualitative differences. In underfeeding for example, a deficiency of energy may often be accompanied by deficiencies of protein, minerals, or vitamins, while high plane rations are often poorly balanced. From the many reports of experiments on small laboratory animals it is now clear that severe inanition will have a markedly adverse effect upon the development of the reproductive organs and the production of semen.

Less severe inanition in rats, while not influencing testicular morphology, may suppress male sex hormone production with resultant atrophy of the prostate and seminal vesicles (Moore & Samuels, 1931). Experimental work involving cattle has also shown that a low plane of nutrition may delay the onset of maturity in the female (Reid, 1953; Crichton & Aitken, 1954) and reduce the growth rate and quality of semen in males (Jones & Dougherty, 1945; Flipse *et al.*, 1953). A study on monozygous twin bulls of the residual effect of different rearing intensities up to 18 months of age has shown that such differences had relatively little effect on the bull's semen production and serving ability when compared with the variations between the twin pairs (Bane, 1949, 1954). James (1950) studied the effects of high and low planes of nutrition from 20 weeks to two years of age in an experiment with five pairs of identical twin bulls. At two years the body weights of animals in the low plane group were approximately 60 per cent of those in the high plane groups. Exhaustion tests showed that the semen production of bulls in the low plane group was considerably depressed although there had been no deterioration in spermatozoan morphology.

In mature bulls changes in the level of feeding have relatively little effect upon sexual function. Branton *et al.* (1947) found no differences in semen production and fertility between bulls which were fed at total digestible nutrient intake levels of 100, 120, and 140 per cent of Morrison's recommended maintenance requirements for dry dairy cows. Mann & Walton (1953) showed that the volume and density of semen and the motility of the spermatozoa were not significantly changed in a bull whose level of feeding was so reduced that during a period of 23 weeks its body weight fell by an average of 6·5 kg. per week.

It is sometimes assumed that a high plane of nutrition will also have a depressing effect upon reproductive capacity, since the fatness associated with a high condition is believed to depress semen quantity and quality. There is no experimental evidence to support this belief; indeed, on the contrary McKenzie & Berliner (1937) found that rams in show condition continued to be fertile, and that the increased adiposity which is supposed to cause an increased body temperature and associated degenerative changes in the testes did not increase either rectal or scrotal temperatures. However, the traditional adage "fit but not fat" is doubtless a wise one for the "paunchiness" generally associated with fat bulls makes semen collection awkward and it is often difficult to preserve the feet of over-fat bulls in a sound condition.

FEEDING STANDARDS

It is customary to consider the nutritional requirements of an animal under two headings. The *maintenance requirement* represents the amount of food needed to preserve the animal in a state of good health and at the same time maintain a condition of nitrogen and carbon equilibrium. The *production requirement,* on the other hand, is the amount of food needed to produce the body weight increase, milk, wool, eggs, or work expected of any individual animal. The composition of the substance produced reflects, in general terms, the nature of the nutrients that must be supplied to the animal in its food.

In the case of mature bulls the production ration merely has to satisfy the specific requirements of semen production which, when one considers the minute dry matter content of the product, are clearly very small indeed. The nutritional requirements for spermatogenesis have been reviewed by Walton (1949) who summed up the position in these words:

> "In terms of output the testis exerts no special demands upon the general economy of the body and no exceptional substances are formed. We may expect from this that, provided the animal receives a nutrition adequate to maintain bodily health, the needs of the testes will be adequately met".

The now famous work carried out by Brody (1945) at Missouri has shown that the energy requirements for basal metabolism may be represented by the equation:

$$\text{Energy requirement for basal metabolism (Cal.)} = 39{\cdot}5\ W^{0{\cdot}73}$$

(where W is the live weight of the animal in pounds). It is also known that the maintenance energy requirements of mature animals at air temperatures above the critical temperature are about twice those of basal metabolism and therefore the energy needed for maintenance equals $2 \times 39{\cdot}5W^{0{\cdot}73}$ Cal. which, if it is assumed that 4 Cal. are provided by 1 g. of digestible nutrients, may also be expressed as:

$$\frac{2 \times 39{\cdot}5W^{0{\cdot}73}}{4 \times 450} \text{ lb. T.D.N.}$$

This means that for bulls of 1,000 and 2,000 lb. live weight the theoretical energy requirements for maintenance are respectively 6·9 and 11·2 lb. total digestible nutrients per day. The maintenance protein requirements are derived from the work of Smuts (1935) and Brody (1945). The experiments of Smuts showed that the endogenous loss of nitrogen under basal conditions is approximately 2 mg./Cal. For average protein the multiplication of the percentage of endogenous nitrogen loss by 6·25 gives, with sufficient accuracy, the quantity of body protein catabolized. On the basis of the work of Smuts together with an appreciation of other feeding standards, Brody has quadrupled the catabolized protein to give the corresponding necessary intake of digestible protein for maintenance. The suggested standard is therefore, $2 \times 6{\cdot}25 \times 4$ mg. of protein for every Calorie of basal metabolism and $2 \times 6{\cdot}25 \times 4$ mg. for every two Calories of maintenance energy. This works out at 0·1 g. of digestible protein per unit g. of total digestible nutrients. Thus for bulls weighing 1,000 and 2,000 lb. the maintenance protein requirements are 0·69 and 1·12 lb. digestible protein per day respectively. Because these figures are theoretically calculated they are at the best only to be thought of as very general guides to food requirements. However the many practical trials which have now been reported show that on the whole these figures are reliable and the well known feeding standards of Armsby (1917) and of Morrison (1948) correspond quite closely. Morrison (1948) estimates that the minimum maintenance requirements for a 1,000 lb. cow are 7.0 lb. total digestible nutrients and 0·6 lb. of digestible protein daily, figures which do not differ substantially from those calculated above and which in practice have proved to be satisfactory.

In this connexion the work of Branton *et al.* (1947) which has already been mentioned, deserves to be studied. In a well designed experiment these workers fed to breeding bulls (Holstein-Friesians and Guernseys) digestible nutrient levels of 100, 120 and 140 per cent of the Morrison dry cow maintenance requirements. As stated above they found that neither the quantity, nor the quality of the semen produced, nor the fertility of the bulls was related to the digestible nutrient intakes used. In the same experiment they used concentrate mixtures containing 12, 16 and 20 per cent total protein and, as already mentioned, they found the 12 per cent level to be quite satisfactory. There were however, some important differences in body weight among the bulls on the different planes of nutrition, those receiving 100 per cent of Morrison's standard losing a mean of 46 lb. in 90 days, while those

receiving 120 and 140 per cent of the same standard gained over the same period 43 and 102 lb. respectively. With this experience in mind these workers have proposed a set of standards for feeding bulls at A.I. centres in which a 2,000 lb. bull should receive 15·6 lb. digestible nutrients per day instead of the 11·2 lb. which has been calculated theoretically. These suggested allowances of Branton *et al.* (1947) have been adopted in the official recommendations for the feeding of dairy bulls by the National Research Council of the U.S.A. (1950) and an abbreviated version of them is given in Table 1.

Table 1

American Feeding Standards (after Branton *et al.*, 1947) and Tentative Suggestions For British Feeding Standards For Mature Dairy Bulls

Daily allowances per animal in lb.				
	American		British	
Body weight lb.	*T.D.N.*	*Digestible Protein*	*S.E.*	*P.E.*
1200	10·3	1·00	6·8	0·72
1600	12·9	1·20	8·4	0·96
2000	15·6	1·45	10·0	1·20
2400	18·2	1·60	11·6	1·44

In Great Britain no similar feeding standards for bulls have been evolved and because of different conventions (starch and protein equivalents instead of total digestible nutrients and digestible crude protein) it is difficult to apply American experience. However, American laboratory and field experience has upheld the theoretical argument that a liberal maintenance ration will by itself provide an adequate plane of nutrition without a production allowance. The British maintenance standards for cattle, which were advocated by a Ministry of Agriculture Departmental Committee (1925) and are now widely practised do themselves contain a safety margin above the theoretical requirement and are therefore probably quite adequate for the maintenance of health and production of bulls in A.I. centres. These standards are also shown in Table 1 and it is to be hoped that their adequacy will soon be tested experimentally in Great Britain. A recent survey of feeding practices in all A.I. centres in England, Scotland, and Wales (Tribe & Cumming, 1954, unpublished data) showed that in no case were bulls being fed according to a theoretical standard and that all of the planes of nutrition were probably unnecessarily high, generally twice as much protein being fed as is theoretically necessary.

CONSTRUCTION OF SUITABLE RATIONS

As with rations for other farm stock, it is usual to mix a proportion of cereals (high in energy and often home-produced) with a quantity of extracted oil seed cakes or animal by-products (high in protein and usually imported) and to feed the resultant concentrate mixture together with some more bulky food (high in fibre and usually home-produced).

As far as cereals are concerned, oats, barley, wheat and maize are all satisfactory, except that their mineral contents may vary within wide limits and their calcium contents particularly are usually very low. In view of the demands of the human population wheat is seldom fed to bulls, but its milling by-products are readily available. Wheat bran is high in phosphorus and magnesium but contains little calcium and must therefore be mixed with foods having a high calcium content in order to form a balanced diet.

The type of high protein food to be included in the ration has already been discussed and it is sufficient to mention here that, if other components are correctly proportioned, animal proteins have no advantage over vegetable proteins. Since animal proteins are the more expensive the vegetable proteins are usually to be preferred for use in A.I. centres. It is customary for the concentrate part of a bull's ration to consist of several different constituents. This practice has probably arisen from the sensible belief that a complex mixture is more likely to cover every nutritional contingency and so avoid deficiencies, particularly of a trace nature, than a simple mixture. However Reid *et al.* (1948) have compared the value for breeding bulls of a balanced complex concentrate mixture with that of a balanced simple concentrate mixture, and have reported that over a period of 442 days there was nothing to suggest that the one diet was better than the other, except that the simple ration was much the cheaper of the two. The diets used were as follows, and were fed to both groups at the same level of T.D.N.

Ingredient	*Simple Mixture*	*Complex Mixture*
Ground Yellow Maize	54·0	10·0
Beet Pulp	25·0	-
Maize Gluten Meal	10·0	-
Cane Molasses	10·0	10·0
Linseed Meal	-	12·0
Soya Bean Meal	-	17·0
Crushed Oats	-	25·0
Wheat Bran	-	10·0
Dried Alfalfa	-	10·0
Limestone	-	2·0
Iodized Salt	1·0	1·0
Bone Meal	-	0·7
Brewers' Yeast	-	1·95
Mineral Salt Mixture	-	0·1
Fish-Liver Oil	-	0·2
Irradiated Yeast	-	0·05
	100	100

The commonest and cheapest of bulky foods is grass. There is a belief among farmers that pasture provides an excellent food for bulls and may be fed at the height of the grazing season to the exclusion of all other foods. When a supplement becomes necessary, then a cereal food is all that is required. Experimental work carried out by Branton *et al.* (1953) supports this belief, and these workers have

concluded from the statistical analysis of their results that "pasture consisting of of good combination of grasses and legumes is ideal feed for dairy bulls from the standpoint of sexual behaviour, semen production, fertility and economy". In this work each bull was enclosed in an area almost an acre in size and thus was offered grass *ad libitum*, but in spite of this there was no evidence that too much grass made the bulls "paunchy" or clumsy and slow at service. The mixture with which the grazing was supplemented was 40% yellow maize meal, 40% ground oats, 10% wheat bran and 10% cottonseed meal, to which had been added the following mineral mixture at the rate of 2 lb. to 100 lb. of food: 50 per cent salt, 25 per cent oyster shell flour, 25 per cent steamed bone meal. These results are in contradiction to some reports that have come from Russia (Smirnov-Ugrjumov & Laptev, 1938; Laptev, 1940) which suggest that the feeding of grass alone to bulls causes an immediate decrease in sexual activity, with a decline in the amount of ejaculate and spermatozoan concentration 3 to 5 weeks later. Until further evidence is presented it would be wise to treat this claim with reserve.

As grazing lands are not available at many A.I. centres, and the grazing season is of only limited duration, conserved pasture is often of more importance in bull feeding. Hay is well-recognized as an excellent, and almost inevitable constituent of bull rations, but its nutritive value will vary widely according to the conditions under which it is made. Too much hay should not be fed to a bull since the danger of "paunchiness", with the resultant difficulty of semen collection or service, must be avoided. It is therefore common practice to feed 15–25 lb. of hay daily, providing something like 6–8 lb. starch equivalent and $\frac{3}{4}$–1 lb. protein equivalent according to body weight, and to make good the nutrient balance with a supplement of concentrates.

Dried grass is a very suitable food for bulls, and generally has a much higher nutritive value than hay. Unfortunately, however, the composition of the dried grass products on the market is variable and the cost is usually a factor which limits its use. Since some 100–150 acres of grass are needed for the economical operation of one grass drier there are few A.I. centres where the installation of a drier would be an economic investment.

Except for frozen grass, which is not generally available, the only other conserved product is silage which is in many respects the best winter bulky food for bulls. Its main disadvantage is that it must be home-produced and therefore cannot be purchased and stored by A.I. centres which have little or no land attached to their buildings. There is a common prejudice against the feeding of silage to bulls on the grounds that it may impair the libido or fertility, or that it may increase the paunch girth. A survey of American artificial breeding associations in 1949 (National Association of Artificial Breeders of Dairy Cattle, 1949) showed that silage was only fed at 20 per cent of the centres and early recommendations restricted silage intake to 15 lb. per bull daily (Dawson, 1924; LaMaster, 1931). However recent experimental work has shown that very much higher levels may be fed with absolute safety. For example, Quicke *et al.* (1950) compared the performance over a 12-month period of 20 bulls ranging from 14 months to 11 years old and fed the following two diets. Based on the daily intake of a 2,000 lb. bull, group one received 5 lb. of a concentrate mixture (ground maize and oats, wheat bran, linseed cake meal, soya bean cake meal, bone meal, blood meal, tankage and mineral salt mixture), 15 lb. of grass legume silage and good quality hay *ad libitum* (estimated hay intake of 25 lb.). Group two received 45 lb. grass legume silage and 25 lb. of the hay with no concentrates. From the results obtained it was found that, regardless of the ration fed, the initial motility, density and volume of ejaculates were similar, as well as the longevity of the semen in storage. The "over-all condition" and health of the bulls was maintained on the all-roughage diet, and excessive "paunchiness" did not develop despite the feeding of silage at high levels. It was concluded that the two rations were equally efficient in maintaining the reproductive capacity of bulls.

Flipse *et al.* (1952) have confirmed and extended these observations. These workers paired 12 bulls (Holsteins, Guernseys and Jerseys) and, on the basis of 2,000 lb. live weight, fed one of each pair 8 lb. of hay and 15 lb. of a concentrate mixture per day, while the other one received 8 lb. of hay and 80 lb. of silage. The T.D.N. intake per unit of body weight of all bulls was the same and corresponded to the levels advised by Branton *et al.* (1947). The experiment lasted for one year, at the end of which time it was found that there was no significant difference between the two groups in respect of their changes in body weight, heart girth, paunch girth, and height at withers. Similarly, semen quantity and quality tests which were carried out weekly revealed no differences between the two groups which were of statistical significance. On the basis of these results, the authors recommended that a bull may be fed grass silage at rates up to 4 lb. per 100 lb. body weight per day without harmful effects upon either his physical condition or the quantity or quality of semen he produces.

Summary and Conclusions. The influence of various vitamin, mineral and trace element deficiencies upon the reproductive efficiency of male animals has been discussed and the general conclusions drawn that (a) such specific deficiencies are not likely to occur in the diets normally fed to bulls, and (b) even if such deficiencies do occur they usually cause other and more immediately obvious symptoms before fertility is seriously decreased.

There is no good evidence to suggest that the type of dietary carbohydrate or fat influences male ruminant fertility. Evidence that animal protein is essential for the maintenance of fertility in bulls has been contradicted and can no longer be accepted as reliable.

The energy and protein requirements for the maintenance and production of a breeding bull have been discussed and feeding standards quoted.

The foods suitable for inclusion in the rations of bulls have been mentioned and the suitability of cheap, home produced bulky foods has been indicated.

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Latent Virus as exemplified by Mouse Hepatitis Virus (MHV)

BY

A. W. GLEDHILL, M.R.C.V.S., B.A., Ph.D. (Cantab.) AND
JANET S. F. NIVEN, M.D.

National Institute for Medical Research, Mill Hill, London, N.W.7.

A few viruses which cause overt disease in man and animals are known to be also capable of causing infections in which symptoms are manifested only intermittently, often consequent upon some apparently unrelated stimulus. Thus, persons infected with herpes simplex virus may develop local lesions, following infection with a cold, exposure to sunlight, etc. There are other viruses which on epidemiological grounds have been suspected of occasionally causing inapparent infections which on becoming active may be the source of an epidemic. Such a possibility is recognized to occur with the viruses of Newcastle disease and laryngo-tracheitis of fowls and there are grounds to suggest that it might occur with many viruses although the evidence is insufficient to exclude other possibilities. Furthermore, the existence of such inapparent, persisting virus infections of mice has been well established, as for example in the cases of lymphocytic chorio-meningitis, mouse pneumonitis and mouse encephalomyelitis. Since this knowledge has arisen as a result of the extensive use of mice for virus research, a similar situation may exist in the other species of mammals which have been less studied. The most extreme degree of inapparent infection is one in which the virus, although present, is not multiplying. This type of inapparent infection is regarded as latent infection and if, indeed, latent virus infection is a common phenomenon, it would involve epidemiological and immunological consequences of the greatest importance. However, there are two obstacles to investigating latent virus infections: a latent virus cannot be detected without some agent which renders it active; and, even when it had been rendered active, it might be difficult to detect it in the presence of antibodies. The usefulness of mouse hepatitis virus (MHV) as a model for investigating latent virus arises from the fact that mice latently infected with MHV develop active infection if they are inoculated with *Eperythrozoon coccoides*, a normally harmless blood parasite, and from the fact that even active infections with MHV do not readily induce antibody formation. Furthermore, mice, the natural host of MHV, are small laboratory animals in plentiful supply. It is proposed to give an account of the observations and conclusions bearing upon the problem of latent virus resulting from an investigation of MHV infection of mice which has extended over several years and is not yet complete. These conclusions may encourage investigation of latency in virus diseases less amenable to experiment.

SALIENT FEATURES OF MHV INFECTION OF MICE

Interference of MHV with its own growth. MHV produces a mild rarely fatal disease in weaned* mice (strain VS†) which is characterized by

* By "weaned mice" is meant mice aged 20-22 days and just weaned.

† This strain of mice was derived from the virus-susceptible, bacteria-susceptible strain of the Rockefeller Institute mice (Webster, 1937) and bred at the National Institute for Medical Research since 1949.

unusual histological and cytological changes (Gledhill *et al.*, 1955). When mice are infected with a mixture of *E. coccoides* and MHV the virus grows to higher titre and fatal hepatitis is invariably produced (Gledhill *et al.*, 1952; Niven *et al.*, 1952). When mice are infected with the virus followed 2-3 days later by a mixture of the virus and *E. coccoides*, the high mortality associated with the mixture does not occur. This sparing effect of prior infection with MHV alone could not be ascribed to humoral antibody. Indeed, experiments indicate that prolonged hyperimmunization is necessary to produce detectable amounts of antibody (Dick *et al.* (*b*), to be published). Moreover, under favourable conditions the sparing effect was detectable when the inoculation of MHV had preceded that of the mixture by as little as 12 hours and persisted for at least 28 days (Gledhill & Dick, 1955). Prior infection with MHV prevented the virus in the presence of *E. coccoides* from attaining its usual high titre, whereas it did not interfere with the multiplication of *E. coccoides* (Gledhill *et al.*, 1955). It was inferred that infection with MHV evoked tissue resistance to its growth in the presence of *E. coccoides* and this tissue resistance accounted for the relative mildness of MHV infection of weaned mice. In mice previously or simultaneously infected with *E. coccoides*, the tissue resistance was not evoked, thus permitting the virus to grow to a higher titre and produce fatal hepatitis. In unweaned mice the virus grows to high titre in the absence of *E. coccoides* and produces fatal hepatitis. On one occasion an MHV-like agent (MHV-3, termed "Craig" in earlier publications) was isolated from the VS strain of mice and it differed from MHV chiefly in its capacity to attain high titres and produce fatal hepatitis in weaned VS mice in the absence of *E. coccoides* (Dick *et al.* (*a*), to be published). Presumably, MHV in unweaned mice and MHV-3 in weaned mice grow to high titre because their growth is not checked by tissue resistance and, as might be expected, the titre of neither virus is increased by the presence of *E. coccoides*.

Liberation of MHV from carriers by E. coccoides. At autopsy 10 days after infecting weaned VS mice with *E. coccoides*, about 10 per cent show severe or even fatal hepatitis. Suspensions of the livers of such mice produce fatal hepatitis when inoculated into VS mice of similar age and the effect is serially transmissible. This is due to the presence of both *E. coccoides*, derived from the original inoculation of this parasite, and MHV, carried by the mice which developed hepatitis following inoculation of *E. coccoides*. The virus may easily be freed from the parasite by filtration through a Gradocol membrane of 250 mμ and in other ways. Virus suspension can then be passed serially at weekly intervals producing the mild, characteristic MHV hepatitis and regularly producing fatal hepatitis whenever it is recombined with *E. coccoides*. A variation of this type of experiment consists of infecting 5 or 6 weaned mice with *E. coccoides* and passaging liver-spleen suspensions serially at weekly intervals in similar numbers of mice. Between the 2nd and 5th passage the infected mice develop hepatitis. If, however, such passages are carried out in the absence of *E. coccoides*, the liver suspensions do not contain MHV, as may be readily shown by inoculating them into mice pre-treated with *E. coccoides*. In fact, MHV was demonstrated only on one occasion from liver-spleen suspensions in more than 60 passages using about 400 normal weaned mice (Gledhill & Dick, 1955). It was therefore inferred that the virus was latent in carriers and that it was activated by infecting them with *E. coccoides*. Another observation confirmed the latency of MHV in carriers. By determining the MHV carrier rate to be 10 per cent in 100 mice from a single sample of 200 mice and by showing that all of the remaining 100 mice succumbed to the lethal action of the mixture, it was possible to conclude that carriers are not

resistant to it (Gledhill & Dick, 1955). Yet, as has been stated, experimental infection of mice with MHV rapidly induces resistance to the fatal action of *E. coccoides*-MHV mixtures. It appeared that the virus present in carriers had not multiplied significantly in them.

Other stocks of mice have been shown to carry MHV. These include the P*, C57† and C3H† strains (Gledhill & Dick, 1955) and also a stock of mice‡ free from mouse encephalomyelitis, *E. coccoides* being necessary for facilitating these isolations except in the case of the C3H strain. In early experiments with this strain of mice MHV was isolated several times in the absence of *E. coccoides* although some months later the presence of the parasite was necessary for the isolation of virus (Gledhill & Dick, 1955).

As a result of the ubiquity of MHV in mouse stocks, the maintenance of *E. coccoides* by passage in mice presents the problem of keeping extracts containing it free from contamination with MHV liberated from sporadic carriers. This problem was overcome by diluting at each passage the tissues containing the parasite 10^7- or 10^8-fold, both for making the subsequent passage and for infecting mice as a preliminary to experiments with MHV. Under the conditions which obtained the infectivity of any virus present would be lost at a 10^3- or 10^4-fold dilution.

Viruses related to MHV. The relationship between MHV, the hepatitis virus described by Nelson (1952) and JHM virus described by Cheever *et al.* (1949) was described in a preliminary communication by Gledhill *et al.* (1953). In a further publication (Dick *et al.*, (*b*) to be published), the authors suggest that MHV, Nelson's virus and JHM be called MHV-1, MHV-2 and MHV-4 respectively, leaving MHV-3 for the virulent MHV-like agent (formerly called Craig) recovered from the VS stock of mice, as already mentioned. Two other viruses (H747 and EHF120) isolated from Japanese mice have been shown to be antigenically related to MHV and JHM (Morris, 1954) and probably belong to the group. It would appear that MHV is a member of a group of murine viruses having many common properties of which the following are notable :—

(1) They occur in latent form in mouse stocks.

(2) They are potentially both hepatotropic and neurotropic.

(3) They produce qualitatively similar histological and cytological lesions.

(4) Their pathogenic action in the liver is enhanced in the presence of *E. coccoides*.

(5) Their host range is similar (mice; cotton rats and hamsters susceptible to intracerebral inoculation of neurotropic variants produced in mice).

(6) They are similar in size and heat lability.

(7) Although they evoke a poor antigenic response, they appear to be related antigenically.

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* A strain of white mice bred at the National Institute for Medical Research since 1922.

† Obtained from the Imperial Cancer Research Fund Laboratories by the courtesy of Dr. J. Craigie, O.B.E., F.R.S.

‡ A few breeding mice free from TO strain encephalomyelitis were kindly supplied in October 1953 by Dr. H. von Magnus of the State Serum Institute, Copenhagen (von Magnus, H., 1950) and have since been bred at the National Institute for Medical Research, Mill Hill.

A hypothesis explaining the salient features of MHV infections. Gledhill & Dick (1955) advanced the following hypothesis to explain the facts cited above. Inoculation of MHV into a mouse produces active infections of some liver cells and latent infections of other cells. By active infections of cells is meant infections in which cells liberate virus and are probably destroyed; and by latent infections of cells is meant infections in which the cells do not forthwith liberate virus but become resistant to subsequent infection. As a result of producing latent infections in many liver cells MHV causes only a mild and regressive hepatitis. The enhancing effect of *E. coccoides* would then be due to its suppressing latent and promoting active infections of cells. The power of *E. coccoides* to produce a progressive MHV infection in carriers could not be due to its converting latent infections of cells into active ones, since if it could do this prior inoculation of MHV would not protect against the fatal effect of MHV-*E. coccoides* mixtures. The alternative to direct activation of latent infections of cells by E. *coccoides* is that latent infections can spontaneously become activated with liberation of virus and that this liberated virus then produces latent infections of neighbouring cells in the absence of *E. coccoides* and active infections in its presence. Evidence that latent infections of cells do spontaneously become active is implicit in the results recorded in the succeeding paragraph.

Further evidence has recently been brought in support of the hypothesis (Gledhill & Dick, to be published). It has been shown that the dose of MHV suspension infecting 50 per cent of mice (ID50) is significantly higher when titrated in mice previously infected with *E. coccoides* than in mice simultaneously infected with *E. coccoides,* in which the effect of *E. coccoides* is inoperative during the earlier stages of virus multiplication. The results show that in the effective presence of *E. coccoides* about twice as many virus particles set up active infection as in its absence. In a further series of experiments, evidence has been obtained confirming that experimental inoculation of MHV produces latent as well as active infections of liver cells and that these latent infections do become active infections much more readily than do the latent infections of cells in carrier mice. In principle, this was achieved by showing that a small dose of MHV (less than one ID50) makes a substantially higher proportion of mice resistant to challenge with MHV-*E. coccoides* mixture some six weeks later than would be expected from an accurate estimate of its infectivity for mice simultaneously infected with *E. coccoides.* These results taken together strongly support the view that MHV produces a proportion of latent infections in weaned VS mice which may later become active infections and that the effect of *E. coccoides* is to facilitate active infections of cells rather than to convert established latent infections into active ones.

Experimental production of carriers. Gledhill & Dick (1955) suggested that in carriers MHV might remain latent on account of the smallness of the infecting dose of MHV under natural conditions; or its non-progressive character might be due to the production by natural MHV of a higher proportion of latent cell infections than is produced by ordinary MHV which has been passaged with *E. coccoides* in the process of isolation and afterwards. In view of the frequency with which latent infections produced by ordinary MHV become active, as shown in the experiments outlined above, it does not seem at all likely that MHV, present in a few cells of carrier mice, could fail to produce evidence of hepatitis in them. In fact, it has proved impossible experimentally to produce carriers by infecting unweaned mice with small doses of ordinary MHV. The majority of survivors of such infection are not carriers when just weaned, although some are found to resist the fatal effect of MHV-*E. coccoides*

mixture. Since it had been observed that MHV isolated directly from a carrier did not regularly produce fatal hepatitis when inoculated together with *E. coccoides,* an attempt was therefore made to create carriers by injecting unweaned mice intraperitoneally with such virus (semi-avirulent MHV), after separating it from *E. coccoides* by filtration. As it produced a high specific mortality in young unweaned mice, 14-day old mice were used for this experiment. When mice which had been injected in this way were inoculated with *E. coccoides* at 21 days of age, about 50 per cent. were found to carry MHV, but differed strikingly from mice infected with ordinary MHV in being fully susceptible to the fatal effect of MHV-*E. coccoides* mixture when challenged 9 days after infection with semi-avirulent virus. However, at 7 weeks of age they were much more resistant to the mixture than comparable controls. This slower development of cell resistance evoked by MHV obtained in this way was believed to be due to slower spread of virus in the liver as a result of its producing more latent and fewer active infections of cells than ordinary MHV produces. By passing ordinary MHV in mature VS mice, suspensions were obtained which failed to produce macroscopically observable evidence of hepatitis even when inoculated into weaned VS mice pre-treated with *E. coccoides.* When such suspensions were injected intraperitoneally into mice 2-4 days old, no hepatitis was noted and the carrier rate at 21 days of age was 33 per cent as compared with 10 per cent for the controls which had received heat-inactivated normal mouse suspensions. Both when just weaned and at 8 weeks of age, these mice were as susceptible to the fatal effect of MHV-*E. coccoides* mixture as the controls. Mice carrying virus were not homogeneously distributed amongst litters. It is worth noting that recent studies (Niven, unpublished observation) suggest that when careful histological study is made of all livers the natural carrier rate of VS mice as determined by means of *E. coccoides* might amount to 25 per cent and if this applies to experimental carriers, the 33 per cent would be raised to over 80 per cent. Although these points are receiving further study, it would seem probable that typical carriers had been produced experimentally, *i.e.,* mice which had been infected with virus so avirulent that the infections of their liver cells were latent with very little likelihood of becoming active. It is intended to publish the details of these experiments to produce carriers.

General assessment of conclusions regarding latent MHV. The evidence would strongly suggest that ordinary MHV produces latent as well as active infections of the liver cells and that latent infections may subsequently become active. Similarly, evidence has been brought to confirm that the effect of *E. coccoides* is to suppress latent and promote active infections. From the experiments directed towards making latent carriers, it would appear that MHV exhibits a graded series of variants from those producing latent infections of cells which are stable in that they do not readily become active infections, to ordinary MHV which produces about 50 per cent of latent infections which are unstable in that they readily become active infections. In the presence of *E.coccoides* avirulent virus produces less stable latent and even active infections of cells and ordinary MHV produces exclusively active infections. If MHV-3 may legitimately be added to the series, it would represent the extreme of virulence, since it produces fatal hepatitis even in the absence of *E. coccoides* and so does not produce any latent infections. No evidence has been brought directly to confirm that latently infected cells are resistant to subsequent infection. The principal facts could equally be explained by supposing that the latent infections which MHV produces in the absence of *E. coccoides* delays the spread of infection among liver cells sufficiently for an unexplained cellular resistance

to develop. Such a cellular resistance might be expected to be due to some kind of interference by imperfect, non-infective MHV particles and the recognized pattern of interference by virus is one of transitory cellular resistance. Yet with MHV, the evidence seems to point to the cellular resistance being permanent. For example, the cellular resistance towards MHV-*E. coccoides* mixtures which reaches a maximum 48-72 hours after injecting MHV alone was undiminished between the 3rd and 10th days and on the 14th and 28th day (Gledhill & Dick, 1955). Since antibody has not been detected in the sera of mice following a single inoculation of MHV (see Dick *et al.* (*b*) to be published), it is difficult to explain the persistence of resistance solely in terms of replacement of waning cellular resistance by antibody. Recently it has been shown that 14 days after infection with MHV, mice remain resistant to the lethal effect of the mixture after 50-75 per cent of their blood has been replaced by blood from normal mice; and normal mice remain susceptible after a similar proportion of their blood has been replaced by blood from mice infected 14 days previously with MHV (Gledhill & Dick, to be published). If the resistance evoked by MHV is not due to antibody nor to ordinary interference, any explanation which may be provisionally proposed must appear unusual.

Gledhill & Dick (1955) noted that the concept of cells carrying latent virus which were resistant to subsequent infections and which occasionally progressed to active infections bore a resemblance to the phenomena associated with bacterial viruses and lysogenic cultures. The existence of a functional similarity would certainly be strengthened if it were possible to confirm that latent infections of cells render them resistant to further infection and, even more, if it were possible to show that latent virus multiplies in step with cell division giving progeny which carry latent virus. The methods so far employed appear inadequate for investigation of these points. However, certain preliminary observations would suggest that the virus can multiply in some ascitic tumours of mice. Following infection, tumour cells at first regress and later commence again to grow, giving rise to tumours which are apparently no longer able to support the mutiplication of MHV (Gledhill, unpublished observation). It is possible that a study of such tumour cells multiplying either in mice or in tissue culture might offer a method of investigating the properties of cells infected with latent virus. If it were found that they resembled lysogenic bacteria in respect of transmission of resistance to daughter cells, they might also resemble such bacteria in respect of convertibility from latent to active infections by appropriate treatments, such as ultra-violet light (Lwoff *et al.*, 1950). However, even if cells latently infected with animal viruses did prove to resemble lysogenic bacteria in transmission of virus to daughter cells, it would by no means exclude the possibility of some essential difference between the two phenomena. For example, the evidence is lacking to confirm the liberation of virus from infected cells by bursting and lysis, as is the case with infected bacteria. Indeed, it has been shown that allantoic cells of the chick embryo infected with influenza virus continue to emit infective particles for many hours (Henle *et al.*, 1954).

Ignorance of the epidemiology of MHV infection. No systematic investigation of the epidemiology of MHV infection has yet been undertaken. It will be recalled that MHV infection, at any rate as it occurs in the VS strain of mice, does not manifest itself at all in the absence of *E. coccoides*. Since virus passaged in mature mice is thereby made latent and since progeny become infected congenitally or during lactation, it would seem that they must derive infection, in one way or another, from mature stock and therefore be infected with latent virus. At present the detection of fully latent virus is only possible

using *E. coccoides,* by tedious experiments evaluated on a statistical basis*. An attempt is being made to produce VS mice free from MHV by breeding only from parents which have produced several litters containing no carriers. This is a long-term project which may not be crowned with success. The observation that amongst experimentally produced carriers, the proportion of carriers from litter to litter is not homogeneous suggests that *E. coccoides* activates latent MHV more efficiently in mice of some litters than in those of other litters. Consequently, attempts to select breeding stock giving progeny not carrying MHV may really be the selection of breeding stock giving progeny in which the activation of MHV is more difficult. Such selection would probaly lead to a reduction of demonstrable carriers rather than complete absence of them and the ultimate usefulness of such mice would be doubtful.

APPLICATION OF CONCEPTS DERIVED FROM MHV TO OTHER VIRUSES

Possible importance of latent virus infections as a general phenomena. Our hypothesis regarding MHV includes the following postulates which might be applied to other viruses:

(1) Viruses can cause latent infections of cells from which virus particles may at some time be liberated.

(2) Avirulent strains of virus are strains containing particles producing latent infections of cells which are very unlikely to become active. Virulent strains contain particles which produce only active infections of cells. Intermediate strains contain varying proportions of particles which give stable latent infections of cells, unstable latent infections of cells of varying instability, and active infections of cells.

(3) Cells infected with latent virus are resistant to subsequent infection; but it must be borne in mind that in latent infections, the number of cells thus infected may be insufficient to prevent serious disease of the host following infection with active virus.

If these principles govern the growth of viruses in general, they might provide a more complete explanation of the persistence of virus in such diseases as herpes simplex than its explanation solely in terms of an equilibrium between active virus and antibody. It might also provide an explanation of the location of viruses between epidemics. For example, in influenza, following recovery from illness some cells may be left infected with latent virus and have a very small probability of emitting virus capable of initiating active infections. If a virus particle of such a kind were emitted after immunity had largely waned, it might set up sufficient infection to render the host a source of virus, if not a clinical case of influenza. On the other hand, following active infection with the viruses causing such diseases as rinderpest and yellow fever, the presence of cells infected with latent virus which had a somewhat higher probability of becoming active and liberating virus would maintain humoral antibody indefinitely. Viruses attenuated for use as vaccines would represent variants giving rise mainly to latent infections in the cells of the natural host

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* It is interesting that the P strain of mice which carry both *E. coccoides* and MHV are when just weaned about 70 per cent resistant to the fatal action of MHV-*E. coccoides* mixtures. It has been shown that this resistance is not directly due to the presence of *E. coccoides* in weaned mice and yet if P mice are freed from *E. coccoides,* following generations of mice are fully susceptible to the fatal action of the mixture (Dick et al. unpublished observations). We believe that the resistance of weaned P mice is due to the carriage of MHV in less latent form in the presence of *E. coccoides* carried by the strain.

and would thereby confer a prolonged immunity, as for example, egg-adapted strains of distemper virus and the 17D strain of yellow fever virus. Study of mice inoculated intraspinally with YSK poliomyelitis virus led Cundy & Evans (1954) to the interesting view that the pattern of virus growth in animals which die late after infection is characterized by a long quiescent period, followed by a period of rapid virus multiplication shortly before signs of illness appear. Such may be the usual course of events in the virus diseases with long incubation periods, as, for example, serum hepatitis and scrapie. Possibly, infecting virus particles give rise to latent infections of cells which, later becoming active, result in progressive infection spreading throughout the susceptible cells.

Possible methods of studying latent viruses. The peculiarity of MHV which has made it possible to show that the avirulence of a strain depends on the proportion of latent infections of cells produced by it, is the fact that concomitant infection with *E. coccoides* specifically suppresses such latent infections. No agent which has a similar effect on other viruses has been described. The reason why it has been possible to suspect that cells infected with latent MHV resist further infection is that MHV does not easily evoke antibody and there are other examples of infections with avirulent virus rendering cells resistant to infection with virulent virus. For example, Frankel & Schlesinger (1954) brought evidence for an avirulent strain of mouse encephalomyelitis virus of GA type, present in early passage mouse brain extract, which interfered with the pathogenic action of virulent virus present in later passage mouse brain extract; Morris *et al.*, (1954) showed that nebulized attenuated distemper virus induced resistance in ferrets within 6 hours to challenge with virulent distemper virus; Sabin (1954) observed that in tissue culture a non-cytopathogenic variant of poliomyelitis virus induced resistance to superinfection with a cytopathogenic variant and noted the similarity to lysogenic cultures of bacteria. In looking for systems for investigating other latent viruses, the use of tissue culture and egg embryo techniques would eliminate the complication of antibody production. Moreover, it might be easier to find agents which influence the proportion of latent infections in tissue culture than in intact animals and latent viruses in tissue culture would probably yield results more quantitative and compelling than is possible with MHV in mice.

Knowledge of the behaviour of latent MHV and any other viruses which had been investigated in detail from this angle would facilitate interpretation of isolated observations in systems less amenable to direct study. Several lines of approach readily suggest themselves and there are doubtless many others. For example, it would be interesting to know at what time after immunization with 17D yellow fever vaccine or with egg-attenuated distemper vaccine antibody concentration was maximal and the extent of its fluctuations over long periods of time. Experiments to activate latent virus might be undertaken in recovered animals after antibody concentration had waned. The mechanism of adaptation of suitable viruses to new hosts might be studied in detail and an obvious choice for this purpose would be influenza virus in mice and hamsters along the lines adopted by Hirst (1947) and Friedewald & Hook (1948). It would be valuable to know what finally happens to infected cells. If active infection does not always destroy them, they might continue indefinitely to liberate virus, or they might throw off infection, becoming either resistant or susceptible to re-infection. More complete knowledge on these points could have an important bearing upon the problem of latent infections. Numerous items of information acquired by such diverse means would probably generate increased understanding of virus growth. Detailed study of an amenable system such as MHV in mice could be of inestimable value as a precursor of stimulating studies in a field offering important possibilities.

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Muscular Dystrophy

BY

K. L. Blaxter, B.Sc. (Agric.), Ph.D., D.Sc., N.D.A. (Hons.) & R. F. McGill, B.Sc.

Hannah Dairy Research Institute, Kirkhill, Ayr.

It is usual to distinguish two major types of reactive process in diseases of the musculature. Myositis is the inflammatory lesion associated with virus, bacterial and parasitic infection, while myopathy is the non-inflammatory degeneration which occurs when infective agents are absent. A third process is atrophic degeneration which is usually secondary to a neural involvement.

Such a classification of muscle diseases is not, however, entirely satisfactory since diseases of entirely different aetiology can produce almost identical changes in the muscle. Thus Zenker's hyaline degeneration, first described in cases of pneumonia and typhoid fever in man, is essentially a non-inflammatory lesion though it can arise during severe infection (Zenker, 1865; Wells, 1927). Conversely, diseases which result from nutritional deficiency or from physical trauma often reveal the presence of those histopathological changes indicative of an inflammatory reaction. In this respect, writing of human muscle disease, Adams *et al.* (1953) stated: "there is no essential difference between the most chronic changes seen in some muscle fibres in chronic forms of polymyositis and the changes that are seen in the more rapid forms of muscular dystrophy." Furthermore, despite many decades of careful study, it is becoming more and more apparent that the diagnosis of the myopathies of man and their separation from polymyositis due to known and unknown causes is extremely difficult indeed (Nattrass, 1954).

The above general remarks are necessary before considering the muscular dystrophies and myopathies of farm livestock since it is not clear from the many published case reports whether or not a disease described in one part of the world is identical with another described elsewhere. Thus diseases have been described in which deviations in the pattern of degeneration are discernible, varying degrees of inflammatory reaction are present, and attempts at repair differ in extent and intensity. While not excluding the considerable possibility that the diseases described represent a number of distinct pathological entities, it is possibly a more profitable approach to regard the reactions of the muscle cell to physiological and biochemical stress as rather limited in extent, and the secondary reaction of the connective tissue and regenerative processes as slightly more variable. This approach necessarily places greater emphasis on the causes of the degenerative process in attempts at classification, and regards the muscular lesion as a sign, possibly common to a number of distinct disease entities. Such an approach makes it difficult subsequently to provide more than a general interpretation of the many isolated case reports which have usually been based on single abattoir specimens. The large number of such reports, however, makes it certain that diseases of the musculature of cattle, sheep, horses and pigs are widespread.

For the above reasons, the present review first deals with the production of myopathy in farm animals kept under experimental conditions, and adduces results obtained with laboratory animals as well. The enzootic muscle diseases are then discussed paying particular attention to those studies which have provided information relating to cause and prevention rather than to those which deal with the detailed pathological description of individual cases. Those interested in the minutiae of histopathological change and the possible multiple nature of the myopathies of farm stock should refer to the many German, French and Italian papers published on the subject, together with the monograph of Adams *et al.* (1953).

THE EXPERIMENTAL PRODUCTION OF MUSCULAR DYSTROPHY

An experimental muscular degeneration, consisting of a generalized myopathy of skeletal and cardiac muscle was first produced by Goettsch & Pappenheimer in 1931 and later shown to be cured by administration of α-tocopherol (Mackenzie & McCollum, 1940). The disease was regarded as a dietary deficiency of vitamin E. Even earlier Evans & Burr (1928) had described a paretic syndrome in rats given diets deficient in vitamin E, and this too was shown to be associated with muscle lesions by Olcott (1938). For such experimental myopathies the term " nutritional muscular dystrophy " was proposed because of the resemblance between the lesions observed and those characterizing progressive muscular dystrophy in man.

Since these early experiments at least 15 species of farm and laboratory animals have been shown to develop comparable abnormalities of the musculature when given similar diets (see Mason, 1951). The rate of onset and severity of the disease have varied from species to species and with the age of the animal as well. Thus severe muscular dystrophy can be produced in calves in less than 30 days (Blaxter *et al.*, 1951), whereas in the monkey, it may be necessary to continue the experiment for as long as two and a half years before the progressive weakness characteristic of the disease in this species occurs (Mason & Telford, 1947).

Nutritional muscular degeneration occurs too in those species in which the predominant signs of tocopherol deficiency are nervous or vascular or involve the fat depots. Thus, in chicks, where the usual clinical syndrome of the disease is an encephalomalacia or exudative diathesis, degeneration of the muscle is nevertheless present (Søndergaard *et al.*, 1952; Dam *et al.*, 1952), while in mink where the usual syndrome is steatitis or non-suppurative panniculitis (McDermid & Ott, 1947), muscle lesions occur also (Mason & Hartsough, 1951). Similarly, in the pig, where diets comparable to the original one of Goettsch & Pappenheimer produce either a brown degeneration of the fat depots (Gorham *et al.*, 1951; Robinson & Coey, 1951), or a massive oedema comparable to the exudative diathesis of the chick (Garton & Naftalin, 1953), muscular lesions may also be found (Adamstone *et al.*, 1949; Garton & Naftalin, 1953). The constancy of occurrence of myopathy in a variety of species and in such a variety of so-called vitamin E deficiency states, especially in young animals, is one of the few consistent features in the study of the physiology of the vitamin.

The diets used to produce these abnormalities have invariably contained lard and cod-liver oil, indeed, since the original experiments of Goettsch & Pappenheimer with their " diet 13," it has been customary to include these ingredients in order to produce vitamin E deficiency. Such deficiencies have only very rarely been produced by complete exclusion of the vitamin from rations not containing a source of unsaturated fatty acids. Cod-liver oil contains appreciable amounts of α-tocopherol (Brown, 1953*a*) and its inclusion in the ration usually increases the total vitamin E intake. Yet, such rations lead to a far greater incidence of muscular dystrophy, which, in turn, can be prevented by the provision of additional tocopherol. The conclusion that factors, identified as the highly unsaturated fatty acids, present in marine oils and lard exert a positive action in producing muscular dystrophy is inescapable.

The presence of unsaturated fat is not the sole factor which modifies the course of the so-called vitamin E deficiency diseases. Dam and his colleagues have shown that variations in the salt and carbohydrate content of chick rations low in vitamin E give rise to a bewildering variety of clinical syndromes, sometimes favouring exudative diathesis, sometimes encephalomalacia (Dam, 1944 *a*, *b*, *c*; Dam & Glavind, 1942). Other anti-vitamin substances have also been suggested, including cresylic esters (Meunier *et al.*, 1947; Meunier & Chenavier, 1949). Tri-ortho-cresyl phosphate certainly depresses the absorption of vitamin E from the digestive tract, but in calves it cannot be regarded as an anti-vitamin in the true sense of the term (Cowlishaw & Blaxter, 1955). The clinical signs of paralysis in muscular dystrophy and tri-ortho-

cresyl phosphate poisoning have some points in common, but the latter is primarily a disease of the nervous system and the muscle cell is normal. Sulphaguanidine has also been incriminated as a cause of muscular degeneration (Ashburn *et al.*, 1942). Specific muscle poisons exist, notably certain quinoline compounds, but it is doubtful whether they are involved synergistically with vitamin E (Hicks, 1950).

There is reason to believe that the muscle degeneration characteristic of " vitamin E deficiency " is not a specific lesion, and that it can occur in other deficiency states. The number of experiments reporting such effects are small, and many of the diets employed have included unsaturated fats and relatively small quantities of vitamin E. The possibility that a deficiency of a dietary essential other than vitamin E may increase the susceptibility of muscle to the effects of unsaturated fat should, however, be borne in mind before such experiments are dismissed. Thus, choline deficiency (Aloisi & Bonetti, 1952; Hove & Copeland, 1954) certainly interferes grossly with tocopherol metabolism as judged by plasma concentrations of the vitamin, and muscular degeneration does occur under these conditions. Again, in the experiments in which cardiac muscle degeneration has been described in vitamin B_1 deficiency in pigs (Wintrobe, 1939), lard and cod-liver oil supplied the dietary fat and no vitamin E supplement was given.

It does appear, however, that severe potassium deficiency can give rise to muscle lesions in dogs (Ruegamer *et al.*, 1946; Smith *et al.*, 1950), rats (Cohen *et al.*, 1948), and rabbits (Hove & Herndon, 1955). These do not differ greatly from those seen in vitamin E deficiency, and the suggestion that differentiation is possible microscopically on the basis of the presence of ceroid pigment (Follis, 1946) is not well founded. Ceroid pigment does not occur for instance in dystrophic calves which respond to vitamin E. It is of interest that one of the early biochemical changes in dystrophic muscle induced by vitamin E deficiency is a loss of cellular potassium, and depletion of muscle potassium may be the common path of action in the two deficiencies rather than an effect on neuromuscular transmission as suggested by Hove & Herndon (1955). In any event, the possibility of potassium deficiency in domesticated livestock is extremely remote, indeed, apart from an account given in a brief abstract, the deficiency has never been produced and described in farm stock other than poultry (Flipse *et al.*, 1948).

Apart from dietary factors, muscle lesions may be produced by ischaemia due to a variety of causes. It is possible that some of the small areas of degeneration found in the muscles of new-born calves (Blaxter *et al.*, 1953*a*) arise in this way, more especially since they appear to be more severe under cold conditions.

In concluding this section, it would appear that to regard the presence of muscular dystrophy as pathognomonic of uncomplicated vitamin E deficiency is not justified. Toxicity of unsaturated fatty acids, faulty balance of the ration in respect of sodium and potassium supplies, and possible deficiencies or excesses of other ration ingredients must be considered. The fact that tocopherol is effective as a prophylactic or a cure has however usually been taken to mean that a deficiency state exists.

Experimental muscular dystrophy in calves and lambs. Muscular dystrophy in cattle was first produced and shown to be prevented by α-tocopherol in experiments by Blaxter *et al.* (1951, 1952). These experiments were confirmed by Safford *et al.* (1954). In lambs, Culik *et al.* (1951) produced the disease experimentally, and later a similar disease was produced by Draper *et al.* (1952). Some earlier experiments by Hjärre & Lilleengen (1936 *a*, *b*) in which the disease was produced by giving rations of oxygenated milk are discussed later.

In view of the remarks of the previous section the diets used in the above experiments are of some interest, and Table 1 summarizes their composition.

Table 1

Rations used to produce experimental muscular dystrophy in calves and lambs

Species	*Author and date*	*Source of fat*	*Severity of dystrophy*
	Experiments with rations containing unsaturated fat		
Calves	Blaxter *et al.* (1951, 2)	Commercial (British) lard	Severe
Calves	Blaxter *et al.* (1953)	Cod-liver oil	Very severe, depending on dosage
Calves	Blaxter *et al.* (1951, 2)	Commercial (British) lard and cod-liver oil	Very severe
Calves	Safford *et al.* (1954)	Molecularly distilled lard*	Very severe (first signs 12-24 days)
Lambs	Culik *et al.* (1951) Bacigalupo *et al.* (1952)	Molecularly distilled lard*	Rapid development — 14-50 days
Lambs	Culik *et al.* (1951)	Commercial (U.S.A.) lard	Slower development — 30-80 days
Lambs	Culik *et al.* (1951)	Commercial (U.S.A.) lard and cod-liver oil	Development during 62-74 days
Lambs	Draper *et al.* (1952)	Molecularly distilled lard and tri-orthocresyl phosphate	Severe
	Experiments with rations containing quantities of tocopherol similar to those above		
Calves	Blaxter *et al.* (1953 *b*)	Arachis oil, no additional tocopherol	No dystrophy
Calves	Blaxter *et al.* (1954)	Commercial (British) margarine, with and without additional tocopherol	No dystrophy
Calves	Blaxter (1954)	Low fat, no tocopherol supplement	No dystrophy

* Molecularly distilled lard contains less than 5 mg. tocopherol/100 g.

Table 1 shows that a common factor besides a low tocopherol content in the diets was the presence of unsaturated fat in the form of lard or cod-liver oil. The second part of the table shows that commercial margarine and arachis oil, which admittedly supply some tocopherol, when given as sources of fat, do not give rise to the disease. Furthermore, unpublished work (Blaxter, 1954) has shown that in a period of three months muscular dystrophy does not occur when rations containing minimal amounts of tocopherol are given, provided they are free from fat. The conclusion that the overt muscular dystrophy syndrome is closely related to the unsaturated fat content of the diet receives additional confirmation from the experiments of Underbjerg (1940) and Thomas & Cannon (1937) with goats, and by Gullickson *et al.* (1949) with cattle. In these experiments rations rendered low in vitamin E content by ether extraction or by treatment with ferric chloride were given. They were sufficient to result in a lowering of the tocopherol content of the tissues, but over the several years of experiment involved, muscular dystrophy was absent. In adult cattle reared from birth on these rations some deaths were attributed to cardiac failure, but the heart lesion described (Gullickson & Calverley, 1946) was not that severe degenerative sequence seen in the experiments with calves and lambs given the diets shown in the upper part of Table 1. Possibly the lesion was comparable, but detailed pathological findings have not been published. Further suggestive evidence that dietary unsaturated fat is an important factor in producing dystrophy is the isolated observation of Marston & Peirce (1942) recording the appearance of dystrophy in sheep confined to experimental rations containing linseed oil. The report on the occurrence of the disease in cattle given coconut meal is possibly also pertinent (McDonnough, 1953).

In view of the practical importance of cod-liver oil in livestock feeding, considerable attention has been paid to its adverse effects. These were first recognized before 1900, but Agduhr (1927) working with a variety of species including man (Agduhr & Stenström, 1929) was the first wholeheartedly to condemn cod-liver oil as a dietary adjuvant. At the time Agduhr's work was very largely ignored or rather overshadowed by the known value of cod-liver as a source of vitamins A and D, and its demonstrable success in the prevention and virtual eradication of human rickets. In the light of such spectacular success in combating a deficiency disease, that cod-liver oil could be toxic, must at the time have been rather difficult to believe.

The apparent toxicity of cod-liver oil to calves was noted by Slagsvold (1925) as well as by Agduhr, but in numerous experiments in the U.S.A. in which cod-liver oil was given to supply vitamin A no mention was made of any injury. Turner *et al.* (1936) did briefly record that calves did not survive when given 2.0 g. of the oil per kg. body weight, without ascertaining the cause, whereas Davis & Maynard (1938) found that no electrocardiographic or other abnormality could be detected. Unequivocal evidence that the addition of cod-liver oil to normal rations produced muscular dystrophy in calves, and that the effect was absent in calves given other vitamin A and D supplements was provided by Blaxter *et al.* (1953*d*). They showed that the addition of 1, 2 or 4 oz. of cod-liver oil to a ration of dried skim milk powder produced severe dystrophy, a particularly high incidence of cardiac lesions being found. Further experiments (Blaxter *et al.*, 1953*c*) showed that in order to prevent the disease when only one fluid oz. of cod-liver oil was given, it was necessary to provide 200 mg. DL-α-tocopherol daily along with the oil; with smaller amounts of oil less tocopherol was necessary. Calves were used in experiments to fractionate the oil (Blaxter *et al.*, 1953*b*). Earlier attempts had been made to do this with mice by Agduhr (1935) and with guinea pigs by Madsen *et al.* (1935). In the latter instance the experiments followed others in which a muscular degeneration had been produced in goats and sheep in attempting to devise "synthetic diets" for herbivorous animals. Cod-liver oil had been incriminated as the causative agent. Unfortunately, the fractionation experiments of Agduhr and the Cornell workers were equivocal, dystrophy occurring with all the fractions tested. In the tests with calves, however, it was shown that toxicity resided in the unsaturated fatty acids and that the non-saponifiable residue and saturated fatty acids were without any effect. Further studies indicated that the more highly unsaturated acids (iodine value 270) were more toxic than the less unsaturated acids (iodine value 110) (Blaxter *et al.*, 1953*b*).

Comparable results of fractionation studies were obtained by Dam (1944*b*) in a study of hog liver fat toxicity in chicks: the anti-vitamin E effect resided in the most highly unsaturated fraction (iodine value 241). Similarly, Dam & Granados (1945) investigating depigmentation of the enamel of rat incisors found it to be induced by a cod-liver oil fraction with iodine value 283. No work has been done to find whether a single molecular species of polyethenoid acid is involved or whether toxicity is a general property of unsaturation.

Many practical instances of cod-liver oil poisoning have come to our attention. They occur most frequently in the spring when the tocopherol reserves of calves are extremely low.

CLINICAL SIGNS OF EXPERIMENTAL MUSCULAR DYSTROPHY

The clinical signs of experimental muscular dystrophy are those of an impairment of muscular function. Such impairment results in compensating changes in behaviour, stance and gait, the animal attempting to minimize the strain on and pain in the affected muscles. The disease invariably produces symmetrical lameness, and can thus be distinguished from lameness due to injury or other cause. The disease is similar in calves and lambs as far as clinical signs are concerned, but is more easily seen in calves, especially in those with a thin hair coat.

Understandably, clinical signs vary according to the particular muscles which are affected, and consequently are highly variable. From a consideration of many experimental cases a rough classification may be made into predominantly skeletal muscle cases, predominantly thoracic and diaphragmatic cases and cardiac cases. Cases involving the skeletal muscles can first be recognized by lethargy, unwillingness to stand, and attempts by alteration of stance to minimize strain on particular muscles. Muscular weakness can be demonstrated in various ways by increasing the strain on the muscles concerned. Within this group, clinical signs include rotating movements of the hocks when walking, spreading of the toes, relaxation of the carpal and metacarpal joints, elevation of the suprascapular margin, inability to raise the head, difficulty in swallowing, inability to use the tongue and relaxation of abdominal muscles. The face of the calf has a drawn appearance. The muscles themselves on palpation are hard and rubbery in texture, and occasionally very swollen. These signs appear quite comparable to those of pseudo hypertrophic dystrophy in man, especially of the scapulo-humeral type. In lambs, a stiff " marching " gait, and difficulties in balancing, are more specific signs observed together with the tendency to lie down most of the time. In very severe cases, animals can stand for only a few minutes when violent tremors are present. These are thought to be an effort tremor not to be confused with tetany.

The diaphragmatic type simulates pneumonia. The intercostal muscles in these instances are dystrophic to some degree and the diaphragm is usually severely affected. Respiration is carried out almost entirely by the use of the abdominal wall.

The cardiac type of dystrophy may be present without clinical signs of dystrophy elsewhere. Cardiac seizures may occur and be recognized, but usually the calf or lamb dies suddenly without any premonitory sign. Auscultation may reveal disorganization of rhythm and other abnormalities. Electrocardiographic investigations have been made in dystrophic lambs by Bacigalupo *et al.* (1953) and by Draper *et al.* (1952) and in calves by Safford *et al.* (1954). In our own studies we have been unable to interpret our findings adequately owing to lack of information on the normal electrocardiogram (ECG) of the calf. The work with lambs and calves indicates a decrease of the P-R interval. The same change was noted by Gullickson & Calverley (1946) in their cattle reared on vitamin E free rations. Changes in overall potential were also found. The change in the P-R interval is probably indicative of slowing of auricular activity, but the absence of information on changes in the ECG in known cardiac abnormalities in cattle, makes interpretation difficult. The electrical axis of the heart in animals differs from that in man and extrapolation of findings from human cardiology is not warranted (Alfredson & Sykes, 1942). What is, however, certain is that in some individuals subsequently found to have severe cardiac lesions, very grossly abnormal ECG's have been found. In view of the variation in the position of the lesion in the left and right ventricles from animal to animal, it appears doubtful to us whether a defect in the ECG would be common to all. It suffices to conclude that profound disturbances may be detected in some animals, and that in others abnormalities are small.

GROSS PATHOLOGY

The experimental lesion of muscular dystrophy has been described in the publications cited in Table 1. It consists of white or greyish areas of degeneration, localized in extent, usually involving a large group of muscle fibres. Sometimes, the area comprises a shell of white degenerate tissue surrounding normal coloured muscle; in other instances, the degeneration consists of a central core. Dystrophy of the diaphragm is particularly striking, since it gives the organ a radially streaked appearance, some groups of fibres being normal, others abnormal. The affected muscle tears easily and is usually oedematous to some degree. On rare occasions the lesion is visibly calcified. We have noted this only in calves with concomitant kidney lesions due to infection (Blaxter *et al.*, 1953 *b*). Changes in the kidneys consisting of cortical pallor have been

noted by Safford *et al.* (1954). Yellowness of the fat was noted in lambs by Bacigalupo *et al.* (1952), but this has not been conspicuous in experimental dystrophy in calves.

The cardiac lesion is of some interest. Areas of degeneration occurred indiscriminately in both sides of the heart in lambs, whereas in cattle, as judged by the distribution of blood in the carcass at death, and the location of the major areas of degeneration, failure of the systemic side of the heart was most common (Blaxter *et al.*, 1953 *d*). Cardiac hypertrophy, oedema, involvement of the atria, diffuse pallor, and calcification have all been observed in experimental cases. Pneumonia is a common associated condition and in most of these cases there is a history of clinical dysfunction of the throat muscles and tongue before death. This secondary pneumonia is in some cases probably the result of inhalation of food and in others of cardiac dysfunction.

As with the clinical syndrome, so the gross pathological findings reveal considerable variation from calf to calf and from lamb to lamb. In one calf the whole scapular suspension may be found to be degenerate, in another this may be apparently unaffected whereas the lumbar muscles are grossly dystrophic. The factors involved in determining the site of the degeneration are not known.

HISTOLOGICAL CHANGES

The sequence of histological changes in the disease in calves has been worked out by MacDonald *et al.* (1952), while the striking nature of the final lesion has been described by many workers. In the studies at Michigan in which curative trials were performed the pattern of regeneration of the muscle under the influence of tocopherol was also studied (Bacigalupo *et al.*, 1952).

The first changes to be observed in the development of the disease are ones which may easily be mistaken for staining artefacts. Small areas in individual muscle cells react more strongly with the haematoxylin eosin stain, giving sections under high power magnification a rather patchy appearance. Breaking of fibres and the fraying of the ends of the myofibrils is also recognized at a very early stage (Blaxter *et al.*, 1953 *d*). Later, some slight interstitial oedema may be detected followed by recognizable swelling and hyaline degeneration of areas of single muscle cells. Coagulation necrosis follows or in some instances a diffuse granulation leading to vacuole formation without segmentation and necrosis takes place. The sarcolemmal tube collapses following coagulation necrosis and the nuclei of the sarcolemma then multiply, effectively filling in the gap left by the loss of sarcoplasm. This proliferation of sarcolemmal nuclei is one of the spectacular aspects of the disease. Very little inflammatory reaction is present, occasionally a few histiocytes and lymphocytes are observed, but the lesion is essentially non-inflammatory. The presence of bruising due to falls of the animal before death can be detected by extravasations of blood in a number of instances. Narrowing of the lumens of capillaries and arterioles as a result of endothelial swelling has been observed by Hadlow (1953). Fibroblastic proliferation has been noted in severe lesions in lambs (Bacigalupo *et al.*, 1952).

The regenerative phase consists of arrangement of the nuclei in rows, an enhanced Feulgen reaction around the central row of nuclei and the eventual development of new fibres. In the phase of concomitant regeneration and degeneration seen in treated dystrophic animals, giant cells and macrophages, removing the detritus of the degeneration may be seen.

Calcification of the lesion is usually central in the cell, involving those areas which have necrosed. It is a feature of the disease that individual fibres are affected, and in a single section examination reveals that it is usual for a single fibre if affected at all to be affected over a considerable length, while a neighbouring fibre might be normal throughout. This finding appears to be an indication at the microscopic level that some fibres are prone to degeneration, just as at the macroscopic level some muscles of the

body degenerate and others remain unaffected. Why individual cells are singled out in this way is unknown.

BIOCHEMICAL CHANGES IN THE MUSCLE

Fairly complete analyses of the muscles of dystrophic calves were made by Blaxter & Wood (1952). Analyses of the ash of the muscles of their experimental lambs have been reported by Bacigalupo & Luecke (1954). The data of Blaxter & Wood are recalculated and summarized in Table 2. Their mineral analyses agree with those of the Michigan workers.

The Table shows that the primary biochemical changes in the muscle were a loss of the contractile proteins, of creatine and potassium and a secondary increase of sodium and nucleic acid. In accounting for such changes a common factor appears to be gross reduction in the rate of free energy capture by the muscle cell. [Free energy capture is a measure of the efficiency of oxidative processes, usually calculated from the number of high energy phosphate bonds (~) yielded/g. mol. of the substrate oxidized.] The loss of glycogen and the accumulation of lactic acid in such dystrophic muscle and in comparable non-experimental degenerations (Ludvigsen, 1954) would be explained in this way. Energy is needed by resting muscle to maintain the integrity of the peptide linkages of myosin, and to keep sodium out of the interior of the cell (the so-called "sodium pump") while creatine as its phosphate acts as a store of the high energy phosphate bonds on which such processes depend. There are several other pieces of information which suggest that muscular degeneration is the response to dissociation of energy capture from the oxidative process. Thus oxygen lack in the tissues produced in calves by anoxic anoxia results in a creatinuria and mild hyaline degeneration as well as the more usual neural signs (Blaxter & Rook, unpublished). Reduction of the oxygen supply to areas of muscle by emboli artificially induced using starch granules or lycopodium powder has in the horse produced a muscular degeneration comparable to that of myohaemoglobinaemia paralytica (Hutyra-Marek; Carlström, 1930). Similarly, cardiac capillary embolism in cattle infected with trypanosomes gives rise to a similar lesion (Fiennes, 1952). Perhaps the occurrence of muscle lesions in pneumonia may be traced to the severe oxygen lack involved (Forbus, 1926). It would, however, be beyond the scope of this review to discuss the metabolic disturbance in degenerating muscle and the biochemical and enzymological studies which have been concerned with the elucidation of the mode of action of tocopherol.

What is of veterinary interest is that the data of Table 2 show that analysis of muscle can be used to establish the fact that degeneration has occurred. The creatine content is the analytical criterion most usually employed, but determination of the ratio of potassium to sodium is equally effective.

Further biochemical tests, which from the experimental studies of the disease appear useful in studies of naturally occurring myopathies, involve qualitative and quantitative studies of the urine. Sometimes the degeneration is associated with urinary excretion of myoglobin (myohaemoglobin), which originates from the degenerating muscle, but in most instances this pigment is present in very minute concentrations only. In adult muscle disease, such as myoglobinuria paralytica (Carlström, 1930) and other so-called types of polymyositis in horses, myoglobinuria is common. This difference in behaviour of the young and adult animal reflects the very large species and age differences in the myoglobin content of muscle. Thus Lawrie (1950) found a 28-fold increase in the myoglobin content of horse muscle with age, and similar effects have been noted with pigs (McMeekan, 1940), sheep (Hammond, 1932) and man (Biörck, 1949). No differentiation of muscle disease on the basis of myoglobinuria appears possible unless age is taken into account.

A similar marked effect of age applies to the interpretation of the creatine excretion, which, expressed as a ratio to the total creatine and creatinine excretion, falls with age (Safford *et al.*, 1954). Nevertheless, an increase in creatine excretion has

been found in both calves (Safford *et al.*, 1954) and lambs (Bacigalupo *et al.*, 1952; Draper *et al.*, 1952). Interpretation is made even more difficult by the fact that the creatinine excretion per unit weight also falls with age. In Table 3, the mean values obtained in the Michigan experiments are given. They show the diagnostic value of the ratio of creatine plus creatinine to creatine. In this respect, it was shown by

TABLE 2

The composition of normal and dystrophic muscle in experimental muscular dystrophy in calves

Constituent	*Normal calves*	*Dystrophic calves*	*Remarks*
Dry matter of fresh muscle %	21	18	Oedema clearly present
Gross composition (% of D.M.)			
Ash	5	up to 14	These changes are in part secondary to the change in the nitrogen-containing fraction
Total lipid	3	up to 5	
Total N	14.4	down to 11.9	Largely due to the fall in globulins and creatine
Ash fraction (mg./100 g. D.M.)			
Sodium	30	up to 124	Loss of intracellular potassium associated with loss of contractile protein. The sodium infiltration is probably due to oedema
Potassium	1720	down to 919	
Calcium	25	up to 200	Due to mild calcification
Magnesium	80	81	No apparent change
Phosphorus	1070	1080	
Lipid fraction (mg./100 g. D.M.)			
Free cholesterol	398	604	Increases probably almost entirely secondary to changes in the protein moiety
Ester cholesterol	57	62	
Phospholipid (as P)	184	238	
Tocopherol	6	9	Note no depletion of the muscle tocopherol
Nitrogen-containing fraction (as N in % of D.M.)			
Stroma	3.2	3.9	Increase mainly due to decrease in the proportion of contractile proteins
Collagen	1.8	3.5	
Myoglobin*	0.018	0.012	A fall
Other globulin + albumen	10.0	6.6	Includes the contractile proteins of the cell
Nucleic acid†	0.17	0.38	Due to sarcolemmal proliferation
Creatine	0.65	0.14	A marked fall

* Calculated from the iron content.

† Calculated from the P content of the nucleic acid using a N:P ratio (atomic) of 3.8.

Blaxter *et al.* (1952) that the excretion of creatinine falls in the disease, an effect they attributed to a reduced muscle mass. The ratio of creatine to creatinine would thus be a more useful index than the one employed by Bacigalupo and his associates, since in their index one component of the numerator (creatine) increases in the disease, and the other (creatinine) falls. This is as apparent in their experiments with lambs as in those with calves. Normal values of the ratio creatine : creatinine are well below 0.7, and in muscular dystrophy increase to above 1.0. In severe cases the ratio may be as high as 5.0.

TABLE 3

The mean daily excretion of creatine and creatinine in experimental muscular dystrophy (Bacigalupo *et al.*, 1952)

		Age of animal (days)			
		0-10	20-30	40-50	60-70
Creatine (mg./day)	Deficient lambs	75	99	142	239
	Normal lambs	53	48	62	107
Ratio creatine + preformed creatinine to preformed creatinine	Deficient lambs	1.55	1.58	1.82	3.28
	Normal lambs	1.39	1.32	1.31	1.44

THE ENZOOTIC MUSCULAR DYSTROPHIES

The above account of muscular dystrophy has been based entirely on the experimental diseases in which tocopherol has been shown to be a preventative or to effect cures. Close similarity of the rations employed to produce the disease possibly precludes maximal variation in the syndrome, but even so considerable variation does exist as evidenced by the diversity of clinical signs. Similar variation may be expected to occur in the naturally occurring diseases, and where the amount of observational material is small the possibility of wrongly placed emphasis in its description must be considered.

ENZOOTIC MUSCULAR DYSTROPHY IN SHEEP AND LAMBS

Diseases of the muscles of sheep have been described from many countries, including those of Europe, N. America and New Zealand. The reports of these diseases indicate that in all probability only one disease is involved and that vitamin E is effective as a curative and as a prophylactic. The first report was probably that of Hobmaier (1925) who described a disease in Estonia, and called it "myodegeneratio hyalinosa calcificans." Oppermann (1936) used the term "enzootic paraplegia," for what was evidently the same disease. Slagsvold & Lund-Larsen (1934) called the disease "myositis," while Swahn *et al.* (1948) emphasized the true nature of the lesion by employing the descriptive term "Zenker's (waxy) degeneration of the muscle in lambs." These authors had no hesitation in identifying the disease they described with those described by earlier workers. In America less attention was paid to the choice of a scientific name, and the practical term "stiff lamb disease" (Metzger & Hagan, 1927; Willman *et al.*, 1940) which was employed has come into general use in America, Canada and Britain (Cotchin, 1948) and in New Zealand (Hartley, 1953). It appears highly probable, however, that the New Zealand disease, occurring as it does in animals on high quality pasture the year round, and usually coinciding with the animals making

long journeys, has a different primary aetiology to the stiff lamb disease of N. America, and the Baltic countries. It is probably more comparable with the " transport disease of calves," which will be described later. Further common names for the American and Baltic disease in sheep exist, notably, the terms " lamb rheumatism," " föt forangen," and " stiff-neck," all of which emphasize the typical appearance of affected animals. It is suggested that the term enzootic muscular dystrophy should replace these older names.

Probably synonymous with the generalized muscular dystrophy with its tendency towards calcification is the disease of the heart muscle described by Norr and by Seifried under the names epidemic heart muscle deformation (Seifried, 1943) and calcification of the heart (Norr, 1938). These have been thought by many to be comparable to the " plötzliche Herztod " syndrome of pigs, and the pathology of the disease as given in a number of reports has been summarized in detail by Nieberle & Cohrs (1949). In view of the experimental findings in nutritional muscular dystrophy, to distinguish enzootic myocardial degeneration as a separate disease entity does not seem to be justified.

The proof that enzootic muscular dystrophy may be prevented and cured by vitamin E was due to Willman and his associates (Willman, Loosli, Asdell, Morrison & Olafson, 1945, 1946) working in America and Swahn, Obel & Wanntorp (1948) working in Sweden. Attempts to cure an outbreak in England (Cotchin, 1948) were not successful for technical reasons. It may be added here that in several " outbreaks " of the disease in older sheep (wintered hoggs) in Scotland in 1955 α-tocopheryl acetate therapy was highly successful (Graham Marr *et al.*, 1955). The above field trials with α-tocopherol suggest that the disease in lambs is in fact comparable to the experimental muscular dystrophy of the lamb. Clearly, however, some reports of the disease have been complicated by the presence of infection. Thus, Lee & Scrivner (1935) described under stiff lamb disease an entity involving swelling of the legs and a purulent bacterial arthritis, in which muscle lesions comparable to those of enzootic muscular dystrophy were present in the heart and voluntary musculature.

Incidence. The incidence of the disease is known to be high in certain areas, but, unfortunately, comparatively little accurate information is available. It is not an economic proposition to salvage carcasses, so few abattoir statistics can be obtained. Even so, Slagsvold & Lund-Larsen (1934) recorded many hundreds of deaths *per annum* due to the disease while in America Willman *et al.* (1934) quoted surveys of 1929 and 1930 in which the incidence was 6 and 18 cases per 100 ewes lambed. Sholl (1939), recorded an overall incidence of 1% in all *post-mortem* examinations made in the years 1923-38. Table 4 summarizes the incidence of the disease over a five year period in Sweden (Swahn *et al.*, 1948).

TABLE 4

The incidence of enzootic muscular dystrophy in post-mortem examinations of lambs made by the Swedish State Veterinary Medical Service

Year	*Total number of post-mortem examinations of lambs*	*% of cases in which muscular dystrophy was present*
1943	63	38
1944	75	32
1945	65	32
1946	104	79
1947	87	51

The Swedish investigators also state that it has not been uncommon for the death rate to be 40-50% in affected flocks. The variation in incidence from year to year which appears in the table is typical of the Scandinavian, and Baltic countries and of N. America.

The disease usually occurs in the first two months of life and is more severe after a dry cold summer than a warm wet one. All breeds and both sexes are affected. The first cases appear shortly after Christmas, and the incidence increases until the ewes and lambs are at pasture. The highest incidence always coincides with the first day or second day at pasture. It should be explained that it is the common practice in Scandinavia where winters are severe to house the ewes in winter and to put them to grass in the spring. Similar systems are in use in the northern States of America, and even where the sheep are only given rough shelter, extensive supplementary feeding is practised.

Clinical and pathological. The clinical appearance of affected animals is closely comparable to that seen in the experimental disease, but Bacigalupo *et al.* (1952) have remarked: "muscular weakness seemed to be the characteristic symptom of vitamin E deficiency whereas muscular stiffness was the typical symptom of 'stiff lamb' disease." Severe weakness of lambs has, however, been commented on by Scandinavian workers, and it does not seem necessary to make a distinction on clinical grounds. It may also be pointed out that all the lambs of Bacigalupo *et al.* tended to be thin, whereas in the field it is usually the most forward lambs that are affected (Slagsvold & Lund-Larsen, 1934; Willman *et al.*, 1940).

The macroscopic findings at *post-mortem* examination are also comparable to those in the experimental disease, the most common sites of the lesion being the infra- and supra-spinatus, the pectoral, neck and dorsal muscles, and the diaphragm. The muscles of the jaw are very rarely affected (*c.f.* the disease in the horse, *vide infra*). Dystrophy of the heart is usually associated with lung oedema. Histologically the muscle cells are buckled and irregularly stained, this initial change being followed by granular degeneration and sarcolemmal proliferation. As in the experimental disease fibroblastic proliferation is seen in severe cases. Calcium salts are deposited in the degenerating cells. Fatty degeneration is never observed. Chemical analyses of affected muscle (Slagsvold & Lund-Larsen, 1934) have shown increases in calcium content, a normal magnesium content, an increase in sodium and a fall in potassium and phosphorus. These changes are in broad agreement with the analyses given in Table 2 for the experimental disease. Whiting *et al.* (1949) demonstrated that the creatine excretion is elevated and can be used to follow the course of the disease and its response to treatment. Again this agrees with experimental findings.

Slow spontaneous remission of the disease often occurs. This is usually associated with atrophy of the muscle, but the complicating factor of inanition is also present. When α-tocopherol is given symptoms disappear in a matter of days. In this respect, it should be pointed out that the treatment of affected lambs may not be successful, owing to the severity of the lesion and secondary pneumonia. Thus, in the experiments of Willman *et al.* (1945) administration of wheat germ oil was not completely successful, nor was treatment with DL-α-tocopheryl phosphate. In view of spontaneous remission, the possibility of secondary infection, and indeed the severe nature of the lesion, curative trials are rather difficult to interpret.

Diet and the disease. The diet which the lamb receives is, of course, colostrum and milk, later supplemented by solid food. The ration of the dam during pregnancy and lactation is therefore of paramount importance. In America, Willman *et al.* (1945) have shown that the disease was particularly associated with a ration of lucerne hay and cull beans, with or without a supplement of oats and barley. It did not occur in lambs whose dams received mixed clover and timothy hay, corn (maize), silage, oats and wheat bran. The addition of wheat bran or wheat germ meal to the "stiff lamb"

ration prevents the disease as effectively as does tocopherol. In Sweden, enzootic muscular dystrophy is associated with winter feeding on hay, straw and roots, together with very limited grain (Slagsvold & Lund-Larsen, 1934). Swahn *et al.* (1948) are of the opinion that the disease is more frequent in flocks which receive hay from improved pasture than in those which consume hay from poor unimproved grazings, and that feeding of concentrated food tends to reduce the incidence. They were unable to detect differences in the vitamin E content of samples of hay from farms with and without the disease. A slightly lower content of tocopherol in liver and muscle was, however, detected in dystrophic lambs as compared with normal unaffected lambs. Whiting *et al.* (1949) have shown that the blood serum of ewes given the "stiff lamb ration" referred to above contained very considerably less tocopherol than that of ewes given a normal ration, and that their colostrum and milk were also very low in the vitamin. They could not, however, detect any very great differences in the tocopherol contents of the rations; indeed, analyses in which the isomers of tocopherol were separated (Brown, 1953 *b*) show that the deficient diet probably contained more α-tocopherol (the most active tocopherol) than the other. Clearly, much investigation is still required to elucidate the interrelationships between dietary tocopherol and other dietary factors.

In any event, it is quite apparent that a major contributory factor in the disease is severe muscular exercise, because the incidence increases very considerably when the ewes and their lambs are released from confinement. Warner (1953) has shown that in a group of 59 experimental lambs whose dams were given rations known to produce the disease, 15 cases of skeletal muscle dystrophy occurred naturally. The remaining lambs were then subjected to forced exercise when a further 16 became clinically affected. On slaughter, only two of the unaffected lambs showed muscular lesions. The trauma induced by exercise, however mild it may be, appears to be a factor leading to the gross exacerbation of the disease in the first few days at pasture.

MUSCULAR DISEASE IN CATTLE

For 70 years or more, degenerations of the muscles of cattle, chiefly of calves, have been known to occur. The diseases were mostly found during meat inspection, and the reports apply usually to only a few specimens, and in these early reports no clinical evidence was presented (Stoss, 1886; Repiquet, 1888; Hüttner, 1893; Faucon, 1897). Flesvik, a Norwegian, appears to have been one of the first to describe the clinical and pathological syndrome in detail in 1896 and he called it "calf rheumatism." In the years that followed, the disease was described in reports from Germany, East Prussia, France, the Scandinavian countries and Switzerland, and there were many polemical arguments as to its cause. These early reports have been summarized by several later workers, notably Vittone (1946) (in Italian) and Hjärre & Lilleengen (1936 *a, b*) (in German). An excellent account is given in the sections "Bewegungsorgane" and "Kreislaufsorgane" in the textbook of Nieberle & Cohrs (1949) and in an earlier article by Nieberle (1926). This early information is not dealt with in detail here.

From a consideration of these and later reports, it appears that, as in the instance of the disease in lambs, many of the published accounts can be regarded as indicative of a single entity, enzootic muscular dystrophy. Other diseases of the musculature not clearly referable to this single entity are the eosinophilic myositis of cattle, which appears to be interstitial and inflammatory (Huchet, 1945), and various rare types of muscular atrophy, probably of hereditary origin (Kidwell *et al.*, 1952; Hutt, 1934). The eosinophilic myositis appears comparable to the eosinophilic myositis of the dog (see Winter & Stephenson, 1952). It is true that the pattern of cause in the hyaline degenerations may differ from case to case, and that in many of these diseases critical experimentation has not been made, but the concordance of evidence suggests that at least three factors are involved in their aetiology. These factors are the presence of

unsaturated fat, insufficiency of tocopherol in the ration or body reserves together with severe muscular exercise or strain.

In recent years, the disease has been described in many countries under different names, and in almost all instances it has been identified with those described by earlier pathologists. It is now known to occur in Finland (Broberg, 1949) where a range of forms of the disease is distinguished, in Sweden (Hjärre & Lilleengen, 1936 *a, b*; Lilleengen, 1941), in Norway (Ender, 1942), in Scotland (Blaxter & Sharman, 1953; Sharman, 1954), in Canada (Schofield, 1953 *a, b*), in Nevada (Vawter & Records, 1947) and in Maine (Houle, 1952) in the U.S.A. It has occurred also in recent years in France (Lesbouyries & Charton, 1947; Querouil, 1950) and in Italy (Vittone, 1946; Mantovani, 1949). Few recent reports have come from Germany, but many of the older ones did, and no reports have been found from equatorial countries or even the southern States of the U.S.A. The disease evidently occurs in Japan also (Goto, 1951).

The names given to the disease are mainly descriptive—" white-flesh," " fish-flesh " or " hen-flesh." Some are based on the district in which the disease occurs, such as " Trondelagen disease "—and others on the general appearance of the lesion, such as " waxy muscle degeneration " or " myositis." The French name is " enzootic paraplegia," the Swedish, " enzootic myositis." Here we would again suggest that the term " enzootic muscular dystrophy " be employed rather than these older ones. Besides the above generalized myopathies occurring under natural conditions, a disease called " transport disease of calves " has been noted in Switzerland and elsewhere, occurring in animals transported by rail over long distances and subjected to considerable muscular strain in the process (Nieberle & Cohrs, 1949). No recent study of this disease has been traced, nor have prophylactic or curative trials been made, but a relationship to enzootic muscular dystrophy is apparent. Similarly, sudden deaths in calves, occurring in the late winter and spring, associated with hyaline degeneration of the myocardium are probably of similar general aetiology rather than separate entities

Prevention and Cure. It is generally recognized that the disease is primarily of nutritional origin: this had been established very early in its history. Which particular nutrient was concerned, however, was not fully established until 1953, when large-scale experimentation in the north of Scotland showed vitamin E to be prophylactic (Blaxter & Sharman, 1953). Before this, dietary deficiencies of vitamin C, the B complex and of phosphorus had been suggested as causes and, in some countries, therapy with sources of these nutrients is still recommended without any controlled test of their efficacy having been made. The basis of these recommendations is given in some detail here.

Hjärre & Lilleengen (1936 *a, b*) and later Lilleengen (1941), described experiments in which the disease was produced as a result of giving three calves milk which had been heated to above 90°C. and continuously aerated for 5 hours. Three control animals received unheated unaerated milk, and a further three the heated milk supplemented with dried yeast. The disease occurred in both the groups that received the heated aerated milk, and it was concluded that since such drastic treatment destroys vitamin C, and since comparable muscle changes occur in scorbutic guinea pigs, the disease was due to vitamin C deficiency. No experiment was carried out in which the heated milk was supplemented with vitamin C. Many experiments have shown that rations which are virtually devoid of vitamin C and which will produce scurvy in those species having a dietary requirement of ascorbic acid are without any effect on calves which can synthesize vitamin C from birth (Thurston *et al.*, 1926, 1929). Furthermore, experiments in which massive daily doses of ascorbic acid were given to calves receiving a dystrophy-producing ration, showed that unlike α-tocopherol, vitamin C was ineffective in preventing the occurrence of dystrophy (Blaxter *et al.*, 1953 *c*). The low stability of tocopherols in the presence of atmospheric oxygen makes it highly probable that in the rations employed by Hjärre & Lilleengen (1936 *a, b*) any tocopherol present

had been inactivated. Changes in the fat may also have occurred. Certainly there is not any proof that the disease is due to a dietary deficiency of vitamin C.

The claim that phosphorus deficiency is related to muscular dystrophy arises from the observations in Norway by Ender (1942) that the hay in the affected areas was low in phosphorus content. Treatment of affected calves with brewers' yeast, yeast ash, sodium phosphate or potassium phosphate produced cures. No information on spontaneous recovery rates in control animals was, however, provided. On the basis of work carried out in 1936-39 Schofield & Bain (1939) reported that in Canada also, phosphoric acid seemed to be of benefit. In a later paper (Schofield, 1953 *a*), it was claimed that the best results were obtained when phosphoric acid and vitamin E were given together, and that the Canadian disease is more severe when the diet is deficient in both phosphorus and vitamin E. Broberg (1949) in Finland was quite certain that phosphorus deficiency was not primarily involved in the disease in his country. It appears probable that phosphorus deficiency occurs coincidentally with muscular dystrophy on the farms concerned. This, in fact, has been admitted by Schofield (1953 *a*). Certainly there is no pathological evidence in other species that a dietary deficiency of phosphorus gives rise to a muscular degeneration of any sort (Follis, 1946). The presence of two nutritional diseases in areas affected by muscular dystrophy has been noted by Blaxter & Sharman (1955). Here the concomitant disease was hypomagnesaemic tetany. Similarly, Broberg (1949) emphasized the high incidence of copper and cobalt deficiencies in the areas concerned in Finland.

The field experiments in Scotland in which vitamin E was given were prophylactic, and therefore do not suffer from the many difficulties of interpretation attending curative trials without concomitant controls. The results are given in Table 5 (Blaxter & Sharman, 1953).

TABLE 5

The incidence of enzootic muscular dystrophy in field experiments in which the prophylactic value of α-tocopherol was tested

	Dam received 1 g. α-tocopheryl acetate daily prior to calving		*Dam received no supplement*	
	Calf received 150 mg. α-tocopheryl acetate daily	*Calf received no supplement*	*Calf received 150 mg. α-tocopheryl acetate daily*	*Calf received no supplement* (NEGATIVE CONTROL)
Total calves	42	42	42	42
Incidence of dystrophy				
Total cases	0	2	3*	11
Deaths	0	0	0	3

* Dystrophy diagnosed before the third day of life, *i.e.*, before treatment of the calf commenced.

These results show quite unequivocally that provided the vitamin E intake of the cow and of her calf is increased, muscular dystrophy does not occur. Further experiments showed that reduction of the daily intake of tocopherol of the calf from 150 mg./day to 10 mg./day did not provide complete protection. These experiments with 78 calves in each group resulted in an incidence of 19 cases in the control group including 3 deaths, and an incidence of 5 mild cases in the experimental group (Blaxter

et al., 1955). It would therefore appear that, as in the instance of the experimental disease in lambs, vitamin E is effective prophylactically in the cattle disease.

Incidence. Incidence statistics are more easily obtained for enzootic muscular dystrophy in calves than they are for the disease in lambs. In a limited area in the north-east of Scotland within a radius of 30 miles around Inverness, the average incidence in calves on affected farms is 20-25% *per annum*. Prior to the extended use of vitamin E preparations, mortality was very high. In Canada, high incidence has also been reported, while in Maine, Houle (1952) reports that " thousands of calves are involved and the heavy losses sustained yearly has constituted a serious economic handicap in the area." Alström (1948) provided the following figures (Table 6) for enzootic polymyositis in a variety of species.

Table 6

Statistics relating to the incidence of enzootic polymyositis (enzootic muscular dystrophy) in livestock in Sweden in the first six months of 1946 (based on reports of all veterinarians (Alström 1948)

	Foals	*Horses*	*Cattle*	*Pigs*
Clinical (treated) cases of the disease Recovered	54	113	80	65
Died	88	90	25	82
Cases of waxy muscle degeneration noted at slaughter (meat inspection)	40	80	74	36

The importance of the disease in Norway and Finland has also been emphasized by the workers concerned.

The disease is primarily a disease of beef cattle which suckle their calves, and the pattern of husbandry with which it is associated is usually the housing of animals during winter on very poor quality food. As with lambs the largest incidence occurs when the calves are released from their winter quarters to pasture in the spring when unaccustomed exercise precipitates the overt syndrome. In Nevada, the disease occurred after wintering on inferior hay, overmature when cut, and as much as 2 years old before consumed. In most instances of the disease in Nevada there was a history of muscular exercise (Vawter & Records, 1947). The description of the Scottish disease by Sharman (1954) emphasizes the turnips and straw diets on which the cows are wintered, and the reduction in incidence which occurs when better quality rations are given.

Clinical and Pathological. The clinical signs of the enzootic disease and of the experimental one are virtually identical. Cardiac, diaphragmatic and skeletal muscle types can be discerned (Sharman, 1954). Other classifications have been suggested such as acute, peracute and chronic. These possibly provide a less informative scheme than sub-classification based on the muscle or muscles affected. Usually, affected animals are slow to gain their feet when in the stall and develop stiff sensitive muscles when turned out. Cardiac cases are particularly common.

The appearance of the carcass is comparable to that noted in the experimental disease. In addition, the lungs tend to be oedematous (Slagsvold & Lund-Larsen, 1934) and the lymph nodes swollen. The kidneys are often slightly swollen too.

Histologically, all the stages of degeneration and regeneration found in the experimental disease can be discerned. The incidence of calcification of the lesion differs in

different areas. It is infrequent in Scotland but fairly common in Scandinavia. Vawter & Records (1947) recorded a calcium content 30 times the normal in one of their cases, and considerable leucocytic infiltration of the lesion in others. Haemorrhages are frequent, and in recovered animals young connective tissue may predominate. In view of our previous remarks on the variability of the lesion as met with under the constant conditions of a laboratory experiment, such variation is to be expected.

MUSCLE DEGENERATION IN HORSES

The various types of traumatic myositis of the horse are well documented in all veterinary textbooks, and are not discussed here, nor are the atrophies of muscle which sometimes follow such injuries. In Sweden in recent years attention has been given to a polymyositis of horses which appears to be closely comparable to that of sheep and lambs (Alström, 1948). Alström makes a classification of the diseases of the muscles of the horse as follows.

(1) *Paralytic myoglobinuria,* exactly comparable to the disease described by Carlström (1930).

(2) *Polymyositis,* a muscular dystrophy comparable to the disease in calves, affecting foals and older horses as well.

(3) *Maxillary myositis* (Käkmyosit) which develops slowly under stall conditions and affects the jaw only.

Broberg (1949) encountered a similar range of diseases in Finland though he classified them differently, separating polymyositis in unweaned colts and lingual myositis in young horses. Sandstedt (1950) described comparable diseases. In America muscular dystrophy was noted in foals by Jones & Reed (1948), who suggested that vitamin E might be involved in its aetiology. In these cases lingual lesions predominated. Carlström & Ottosson (1934) recorded a case of maxillary myositis which later became polymyositic, while in Hjärre's paper (1934), describing a degenerative atrophy of the muscle, it is clear that he regarded the condition as comparable to muscular dystrophy in cattle and sheep, particularly in the the late stages.

In myoglobinuria paralytica (the " Kreuzlähme " of German authors), there is a marked loss of myoglobin from the muscle, giving the urine a dark brown appearance. Concomitantly, there is a 50% fall in the creatine phosphate content of the muscle (Grzycki, 1934) and deficient synthesis of adenosine triphosphate (Grzycki & Gufca, 1937). The lesions present in the muscle are closely comparable to those of muscular dystrophy in other species. Hyaline degeneration is present and a tendency toward calcification has been noted. Nieberle & Cohrs (1949) point out that myoglobinuria occurs also in cattle and that paralytic myoglobinuria is similar to the jaw and tongue myositis already noted.

From such a maze of evidence concerning different types of muscle degeneration in the horse, it certainly appears that they may be of similar aetiology to the muscular dystrophies of cattle and sheep. In particular, their seasonal appearance, their association with sudden unaccustomed exercise, and their rather local nature makes this a particularly attractive generalization. Alström, in fact, suggests that the polymyositis of the horse can be regarded as a muscular dystrophy, and suggests the feeding of vitamin E free diets to produce it and facilitate its study. This has not however been done. Instead, the attempts to produce muscle disease in horses have been varied, ingenious, and perhaps misinterpreted. Carlström (1940), by feeding a ration of polished rice, chaff and casein together with fish-liver oil produced the disease in ten months. He concluded that vitamin B_1 was involved: the anti-vitamin E activity of the fish-liver oil was not considered, nor was intestinal synthesis of thiamin. By feeding hay from a district in which anaemia was common, Carlström & Hjärre also produced

the disease (1938). Earlier, Carlström (1930) had produced sacral paralysis (korsförlamning) by suddenly working horses very hard following feeding on turnips. Turnips are, of course, a major ingredient of the rations of the farms in Scotland where muscular dystrophy occurs in cattle (Sharman, 1954). No further attempts to produce diseases of muscle in horses have been traced.

Sandstedt (1950) made a detailed study of the disease, and was convinced from field studies that over- or underfeeding, variation in protein intake, deficiencies of vitamin A, and of calcium, phosphorus, sodium and chlorine could be eliminated as possible causes. What was of interest was that wheat bran appeared to be protective. In those stables where wheat bran was given regularly the disease did not occur. The only other associated abnormality was a low serum concentration of magnesium. Livers were examined for vitamin A, and for iron, copper, cobalt and manganese and found to be normal.

In view of the prophylactic nature of wheat bran, Sandstedt undertook curative trials. Eleven horses were given vitamin E " in large amounts " with no effect. Unfortunately, no further details were given regarding quantities or the duration of dosage. Treatment with vitamin B_1 was also without effect in sick horses. In a trial with two horses in 1947, inositol had no effect, but in the following year ten horses were treated with inositol and eight recovered. Wheat bran treatment was given as well. Determinations of the inositol content of the muscle did not show any difference between normal and polymyositic horses.

In view of the many difficulties attendant upon curative trials in diseases of the muscle (we have been unable to cure some of our cases of experimental dystrophy in calves), it is difficult to interpret Sandstedt's experiments. No controls were employed in his cases treated with inositol, and, from Table 6 giving the Swedish statistics it is apparent that there is a natural recovery just as occurs in the myopathies of sheep and cattle.

What is certain is that the disease is a very serious entity in Sweden. Alström presents statistics which show that maxillary myositis and polymyositis has an overall incidence in Sweden of more than one case per thousand, and " korsförlamning " accounts for a further 3-5 cases. Horse Insurance statistics for Sweden give closely similar figures. Further experimentation in which vitamin E is used in prophylactic trials is clearly necessary.

DEGENERATIONS OF THE MUSCLES IN PIGS

Experiments employing rations containing fish-liver oil or lard, and little vitamin E have been conducted with pigs and these have shown that a muscular degeneration can be produced, both in the unweaned and in the growing pig (Adamstone *et al.*, 1949; Garton & Naftalin, 1953). The lesion is a hyaline degeneration of the muscle cell, particularly apparent in the heart of the older animal. When given rations deficient in vitamin E and containing unsaturated fat, the pig also develops a massive oedema comparable to the exudative diathesis of the chick (Garton & Naftalin, 1953), abnormalities of the fat depots (Robinson & Coey, 1951), and a " liver dystrophy " (see Blaxter & Brown, 1952-53) comparable to the hepatic necrosis of the vitamin E deficient rat (Naftalin, 1951). The dietary and other factors which operate synergistically with tocopherol deficiency to produce these effects have not yet been completely elucidated.

Under field conditions a disease known as " sudden heart death," " fatal syncope,' " heart muscle degeneration " or " enzootic apoplexy " has been known for a long time. The usual German name is simply " Herztod." It appears to have been first described in detail by Karsten in 1931 (see review by Hupka, 1939) although earlier German textbooks had remarked on it. At first, most attention was paid to the pathological

appearance of the heart. Macroscopically this was pale and patchy or striped, and cardiac failure was indicated by oedema of the lung with or without liver stasis. Macroscopically, the degeneration of the myocardium was a granular type proceeding to vacuole formation or more usually a hyaline degeneration. Inflammatory change was present in some instances giving histiocytic infiltration. Hupka (1939) showed that the muscular damage was not limited to the myocardium: the skeletal musculature was involved also. This was confirmed by Cohrs (1940), and it appears to be generally recognized that fatal syncope is a severe form of "total muscle degeneration" (Ludvigsen, 1953, 1954). In this disease, alterations in the skeletal musculature occur giving it a pale greyish colour resembling chicken or fish meat. The histological changes in this disease include oedema of the interstitial connective tissue, swelling of the fibres, loss of myoglobin and of cross striations. In Dutch cases (Tacken, 1952) hyaline degeneration is predominant.

The incidence of these two diseases has undergone changes. Fatal syncope was very common in Eastern Germany (Rudau, 1939; Maas, 1949) and less common in Western Germany shortly before and during the war years. In a recent article, however, Bolle (1953) stated that with improvement of pig feeding methods the incidence of the disease had been reduced and it had almost disappeared in some areas. In Denmark, Ludvigsen (1954) also indicated that the fatal cases of his "total muscular degeneration" which are synonymous with fatal syncope have declined markedly since 1948. The incidence of the Danish disease is maximal in the hot months of June, July and August; indeed, the fatal cases have usually been ascribed to heat stroke. The German reports emphasize its frequent occurrence in spring and summer, and in Table 6 the cases recorded for Sweden mostly occurred in the spring months. Reports of the Dutch disease have, to our knowledge, been recorded in the last six years only (Postma, 1950). As far as is known, the disease has not been reported in Britain.

In perhaps no other disease of livestock have so many causes been suggested, and indeed, accepted without the test of critical experimentation. The postulated causes make a rather remarkable list. Nieberle (1934) thought fatal syncope arose from a vagal reflex engendered by abdominal pressure. Genetic causes, such as inbreeding and "overimprovement" were the causes mentioned by others (Kurtze, 1944; see also Seifried, 1943). Zinc poisoning was incriminated by Rudau (1939) but, subsequently, experimentation disproved the contention (Schieren, 1946). Similarly, the suggestion that the disease was due to an anaphylactic reaction was disproved by experimentation (Matthias, 1953). Virus or bacterial infection was postulated without any proof (Manninger, 1944; Renk, 1951), but transmission experiments uniformly failed (Maas, 1949). In this respect, however, it should be remembered that severe forms of foot and mouth disease are associated with myocardial degeneration producing the so-called "tiger-heart" (Nieberle & Cohrs, 1949). Possibly this condition is comparable to the Zenker degeneration of cardiac muscle in pneumonia in man where anoxia is a determinant. Complex anterior pituitary dysfunction has also been suggested (Tacken, 1952). Matthias (1953) did in fact produce the clinical signs of fatal syncope by first producing a thyrotoxicosis in the pig with thyroxine, and then inducing ventricular fibrillation with adrenaline. That such drastic measures reflect the underlying causative mechanisms does not seem likely: they are far too severe. Disturbances of heat regulation (Beller, 1944) and mild hypothyroidism (Ludvigsen, 1953) have also been postulated.

What appears reasonably certain from the records of these diseases and the authors' comments is that fatal syncope is associated with a diet largely composed of potatoes and sugar or fodder beets, and little grain or meal of any sort (Hupka, 1939, 1952; Bartel, 1949; Glässer, 1950; Bolle, 1949, 1953; Postma, 1950). The disease has, in fact, been produced experimentally by feeding such rations (Hupka, 1952).

This suggests that dietary factors are involved. Here there is no agreement between authors as to which attribute of the ration is responsible. An excess of carbohydrate, an excess of water, a deficiency of protein, a deficiency of vitamin A or vitamin B_1 (or more usually "vitamin B"!) together with a deficiency of trace elements, and the above in all manner of combinations have been suggested as causes. Vitamins C and E have been suggested as prophylactics (Seifried, 1943). Whether they have been tried in controlled experiments is not known.

Ludvigsen's (1954) experiments on what is evidently the mild form of the disease are of interest. He gave methyl thiouracil to pigs and claimed that he had produced muscle changes comparable to "total muscle degeneration." Iodinated casein (a source of thyroxine) was then given to ten pigs, six being left untreated. Two of the ten pigs given iodinated casein had normal muscles at slaughter, while muscle changes were seen in the remainder. From this work it was concluded that the disease is a "weakening of the pituitary-thyroid and pituitary-adrenal systems." No histological change was, however, found in the thyroid or adrenal glands of pigs in which the disease occurred naturally.

Clearly, there is a considerable need for further critical experimentation to elucidate causative factors in these diseases of the muscles of pigs. Apart from a general statement that nutrition plays a considerable part in the production of enzootic muscular dystrophy and that exercise precipitates the overt condition, no conclusion regarding their aetiology is possible. The fact that many authors have drawn analogies with fatal syncope in pigs and the cardiac types of those diseases of lambs and calves which we have classified as enzootic muscular dystrophy, and indeed with myoglobinuria paralytica in horses, suggests that the nutritional factors involved may be of the same type.

SUMMARY

The above account of the naturally occurring muscular disorders of sheep, cattle and horses, suggests very strongly that many of them have a common aetiology. They mostly occur in countries in which winters are severe so that the animals are housed for long periods. Usually they are associated with poor soils and often with areas where nutritional diseases due to deficiencies of mineral elements are common. Their incidence is invariably at a maximum in the spring or early summer, and generally muscular strain or severe and unaccustomed exercise appears to be immediately responsible for the appearance of overt signs. Nevertheless, histological study shows the disease to be present in a mild form in animals in the areas concerned even before they are allowed exercise, indeed, severe cases do occur without any history of muscular strain whatever.

The fact that the pathological signs of an experimental disease induced by a dietary deficiency of vitamin E or a dietary excess of unsaturated fatty acids simulate those found in the natural occurring ones, provides a basis for supposing that vitamin E is involved in their aetiology. Confirmation of this has been obtained in field trials with lambs and with calves in Scotland, America and Sweden, and there is little doubt that a lack of vitamin E in the winter ration of cattle and sheep is one of the factors accounting for the appearance of the disease in their young. With horses and with pigs, no comparable experiments have been made, but the evidence assembled above suggests that a similar absolute or relative lack of vitamin E in the ration is concerned also in muscular degenerations in these species. Future work will no doubt elucidate this aspect.

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Vitamin A and Ovine Rickets

BY

A. B. GRANT, Ph.D., B.Sc.

Wallaceville Animal Research Station, Wellington, New Zealand.

In recent years much work has been devoted to the study of substances which directly or indirectly interfere with the physiological function of essential nutrients. Of the toxamins and antivitamins which are known to occur in nature, a number can produce deficiency symptoms in livestock. Both thiaminase (Woolley, 1941) present in the raw flesh of some fish, and the bracken-fern factor (Weswig *et al.*, 1946) have induced vitamin B_1 deficiency in animals; the antivitamin K, dicoumarol (Stahmann *et al.*, 1941) is responsible for "sweet clover disease" in cattle and now carotene has been identified as a factor contributing to the onset of rickets in animals fed on green pastures.

The possibility of the presence of a rachitogenic factor in green feed was first postulated by Fitch (1943) to account for the unusually high incidence of rickets in young New Zealand sheep grazing on green cereal crops and good quality pastures. These feeds contained amounts of calcium and phosphorus which were considered more than adequate to meet normal dietary requirements and a simple vitamin D deficiency was considered unlikely since ovine rickets was comparatively rare in far less sunny latitudes than New Zealand. His hypothesis was supported by the fact that Andrews & Cunningham (1945) found the daily vitamin D requirement of young sheep to be considerably less than an amount which failed to protect animals on green-feed oats in a trial conducted by Fitch & Ewer (1944). It was later shown that the fat-soluble fraction from green pasture leaves depressed the bone calcifying effect of vitamin D in rachitic rats and by using the rat as a test animal the active principle carotene was isolated by chromatographic fractionation (Grant, 1951, 1953).

Further work has demonstrated that the rachitogenic factor is vitamin A (Grant, 1954) so that any of the plant carotenoid precursors of the vitamin could produce a rachitogenic effect. Since the field trials of Fitch & Ewer (*op. cit.*) had indicated that supplements rich in calcium and phosphorus were ineffective in reducing the severity of ovine rickets on green-feed, and since the earlier laboratory investigations had produced consistent results indicating a quantitative relationship between the amount of vitamin A administered and its depressing effect on the bone ash response of rachitic rats to vitamin D, it was assumed that vitamin A directly inhibited vitamin D function (Grant, *op. cit.*). More recent work, however, (unpublished) suggests that this is not so, but points rather to an interference with phosphorus metabolism. The question is being further investigated.

The causes of rickets on good green-feeds have been further clarified in recent years by work which has indicated that the green pasture leaf is practically devoid of vitamin D (Thomas & Moore, 1948; Moore *et al.*, 1948; Thomas & Moore, 1951; Thomas, 1952; and Grant, 1951). The relative freedom from rickets of lambs wintered upon more mature feeds and permanent pastures can probably be accounted for by the fact that these usually contain a considerable proportion of dead leaves in which, contrasting with the green, vitamin D concentration is relatively high and carotenoids correspondingly low.

In New Zealand, treatment of rachitic lambs with a single massive dose of 10^6 I.U. vitamin D_2 results in rapid recovery and the same dose provides adequate protection throughout the winter (Ewer & Bartrum, 1948). In Scotland, however, a dose

of 2 x 10^6 I.U. is not sufficient to ensure complete protection for young sheep (Dunlop, 1954). The probably lower winter solar ultra-violet irradiation in Scotland may partly account for the apparent difference in protective dose rate between sheep in the two countries, but other factors may also be involved. The disease in Scotland is frequently associated with "bent-leg" and a calcium deficiency (Elliot & Crichton, 1926), complicating features which are not generally observed in cases of rickets in New Zealand.

Administration of vitamin D in a single massive dose to cover an appreciable period is a convenient method of treatment, but a note of caution might be sounded against adoption of this procedure in all cases. Preliminary results of trials now being conducted indicate that whereas the healthy animal is well able to tolerate a massive dose of 2 x 10^6 I.U. calciferol, in the sick or unthrifty lamb a dose of 10^6 I.U. has a toxic effect manifest in a loss of body weight within ten days.

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Leptospirosis in Domestic Animals, with Special Reference to the Disease in Cattle in Australasia

BY

N. R. McDonald, B.V.Sc., *Veterinary Research Officer, Department of Agriculture, Wallaceville, Wellington, New Zealand.*

Introduction

In the last few years outbreaks of icterohaemoglobinuria or abortion in cattle, pigs and sheep have drawn attention to the widespread occurrence of leptospiral infection. Reinhard (1953 *b*) in the U.S.A. discussed the subject in an annotation. An increasing appreciation of the economic importance of the leptospiroses, especially in dairy cattle, and their transmissibility to man has resulted in diagnostic facilities being made available in many parts of the world and the areas in which the various species are present are being defined.

The progress of this work prompted Ward (1954) to say "Leptospiroses are found whenever and wherever they are sought." One of the commonest in farm animals is *L. pomona* infection. It is likely that leptospirosis has been present for a considerable period in areas where it has recently been diagnosed for the first time.

Isolation of the organism from aborted pig foetuses has been reported (Bryan *et al.*, 1953).

Agglutination titres against leptospira in horses were detected in Switzerland by Heusser (1948) and in the U.S.A. by Yager *et al.* (1950) and attempts have been made to correlate infection with the incidence of equine periodic ophthalmia.

The leptospiroses in dogs have been recognized in Europe and the U.S.A. for many years and are due mainly to *L. canicola* and *L. icterohaemorrhagiae* although other species have been implicated. Mather (1954) citing other workers stated that 11% to 38% of the dogs in some areas of the U.S.A. were temporary or permanent carriers and shedders of leptospira and were of considerable importance in relation to public health. We do not yet know the aetiological significance of the leptospiroses in chronic nephritis of dogs.

THE DISEASE IN CATTLE

Leptospirosis in cattle was reported in the U.S.S.R. by Michin & Azinov (1935), in Australia by Johnson (1943), in the U.S.A by Jungherr (1944), and in Israel by Bernkopf *et al.* (1947). In Australia, New Zealand, and the U.S.A. the disease is commonly caused by *L. pomona*. There is an incubation period of one or two weeks after infection during which leptospiraemia may occur. Reinhard (1953) stated that during this period there is increased erythrocytic fragility, haemolytic anaemia setting in at this stage. Leptospiraemia reaches its peak at the fever stage; there is interstitial nephritis and there are areas of centrilobular necrosis in the liver. The organism may appear in the milk or urine. The destruction of erythrocytes causes anaemia and icterus and if the renal threshold is exceeded, haemoglobinuria. In an

animal that survives, the anaemia is overcome, but the interstitial nephritis becomes chronic and leptospira are shed in the urine for varying periods.

In Adult Cattle. Inapparent infections occur and symptoms range from slight malaise to a severe illness with icterus and haemoglobinuria, and the death rate may be high. Between these two extremes lies a common type of case in which the milk is reduced in yield, is colostrum-like in appearance and may be blood tinged. The animal may remain unthrifty for a considerable period and not regain the previous production level even on the return to normal health. A wide range of symptoms usually occurs in an outbreak but abortion can occur with all forms of the disease. This has been emphasized by the report of Te Punga & Bishop (1953) on an outbreak in which 28% of 88 milch cows produced dead full-term calves or aborted. The cows showed no symptoms and little or no loss of weight, appetite or milk production although 75% were found to have serum antibodies against *L. pomona*.

In Calves. A range of symptoms may be seen in outbreaks in calves, but morbidity and mortality are often high in young animals and they may die quite suddenly showing symptoms for not more than a few hours; haemoglobinuria and icterus, however, usually occur. Some calves may become carriers though showing no symptoms. Te Punga & Bishop reported the demonstration of leptospira in the kidney of an aborted foetal calf and suggested that calves may be infected at birth. This would be of considerable importance in drawing up a scheme for the control of the disease.

IN SHEEP

Hartley (1952) and other workers showed that *L. pomona* infects sheep and can cause the death of ewes and lambs. The importance of the disease in sheep is as yet unknown, but should it be of significance the number of animals likely to be involved will pose problems in treatment and control.

METHODS OF INFECTION

Though the method of spread has not been demonstrated with certainty the urine of carrier animals is a likely source of infection. Cattle may shed the organism for 3 months (Sutherland & Simmons, 1949) and pigs for periods up to a year (Schmid & Giovanella, 1947) and so infection can be perpetuated on farms. The organism can remain alive in ground water for several days under suitable conditions and penetrate intact mucous membranes and minute skin abrasions. It may be possible for infection to spread by contamination of moving water in small streams or open drains.

DIAGNOSIS

Diagnosis is simplified in herd outbreaks if a range of symptoms is shown but it is more difficult in individual cases and in herds where only lowered milk yield or abortion occurs. Serological diagnosis by the microscopic agglutination-lysis test is commonly used, increasing antibody level being indicative of active infection. A complement-fixation test has been developed, but reactions are given for only two to four months after infection and the test cannot be used on the serum of some animals (Reinhard, 1953*a*). The agglutination-lysis test is species-specific and may be employed long after infection. Demonstration of the organism in organs by cultural or pathological

methods is time-consuming. Because animals may shed leptospira for a considerable time after infection, the finding of the organism in the urine of a sick animal does not necessarily mean that symptoms are due to leptospirosis.

TREATMENT

There is relatively little information in the literature regarding the *in vitro* action of antibiotics against leptospires isolated from cattle. There is, however, a considerable amount of clinical data which is difficult to assess since the symptoms of the disease may quickly decline in untreated cases. Though the efficacy of antibiotic treatment is controversial, Brunner & Meyer (1949) found that streptomycin treatment eradicated leptospires from the urinary tract of dogs and hamsters. It was suggested in the Annual Report of the New Zealand Department of Agriculture (1951-52), that streptomycin or aureomycin given in sufficient dosage at the fever stage prevents the development of symptoms and of the carrier state in *L. pomona* infection of calves, and field experience supports this. Any difference in treatment of the acute case and the chronic carrier has not been defined.

IMMUNOLOGY

It has been observed that natural infection confers a long-lasting immunity in animals and this has led to attempts to produce vaccines which would give a solid immunity and prevent the development of carriers. The difficulty of growing leptospires in artificial media, especially virulent strains, has been one of the problems in the production of vaccines. Reports of protection of guinea pigs, hamsters and dogs against infection with *L. canicola* and *L. icterohaemorrhagiae* have appeared in the literature. Brunner & Meyer (1950) stated that the number of inoculations or the antigenic mass is of importance; vaccines would therefore require concentration by centrifugation. They further suggested that a strain of *L. icterohaemorrhagiae* from one area may not offer complete protection against that from another, so that a strain of broad antigenic spectrum is required to produce a vaccine for general use. Some authors believe that a solid immunity is not produced by a chemically-killed vaccine. For this reason York & Baker (1953) who reported on *L. pomona* vaccination, killed the organisms by freezing and thawing. When leptospira is grown on cell-free media it is thought that loss of pathogenicity occurs and these authors believe that the use of a strain which had been maintained on eggs for a number of years may have had a favourable effect upon the antigenic properties of their vaccine. Whether strain specificity is of importance in protection against *L. pomona* it is not yet known; further evidence must be awaited.

When efficient vaccines become available routine vaccination will be indicated in areas where the disease is endemic since, as Reinhard (1953) pointed out, by the time symptoms are manifest, a high proportion of a herd is infected. When infection in the herd is extensive, antibiotic therapy is expensive and it is then too late to vaccinate.

In New Zealand another problem is involved. Most of the losses occur in calves aged 3-6 weeks, and this is the time when seasonal demands on veterinary surgeons are at their highest. Therefore the personnel is not available for large-scale vaccination. In addition, the immunological response of young animals may be low. If the cows were vaccinated it is possible that the colostrum would provide protection, but this has still to be studied. Vaccination of the cows would be more practicable as the work would fall at a less busy season.

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